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(54) Title: LYST PROTEIN COMPLEXES AND LYST INTERACTING PROTEINS

#### (57) Abstract

The present invention relates to complexes of the LYST or LYST-2 protein with proteins identified as interacting with LYST or LYST-2 by a modified yeast two hybrid assay system. The proteins identified to interact with LYST or LYST-2 are 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10. Thus, the invention provides complexes of LYST or LYST-2 and 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, and derivatives, fragments and analogs thereof. The invention also provides nucleic acis encoding the LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10 protein, or derivatives, fragments and analogs thereof. Methods of screening the complexes or proteins for efficacy in treating and/or preventing certain diseases and disorders, particularly atopic diseases, autoimmune diseases, neurodegenerative disease, cancer, pigmentation disorders, platelet dysfunction and viral diseases, are also provided.

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# LYST PROTEIN COMPLEXES AND LYST INTERACTING PROTEINS

This invention was made with United States Government support under grant number 70NANB5H1066 awarded by the National Institute of Standards and Technology. The United States Government has certain rights in the invention.

#### FIELD OF THE INVENTION

The present invention relates to lysosome-associated LYST protein or the novel homolog LYST-2 protein in complex with other proteins. In particular, the invention relates to complexes of LYST protein with one or more proteins chosen from the list comprising: 14-3-3 protein, HS1 protein, Hrs, BMK1 alpha kinase, KB07, Efs, OS9, casein kinase II beta subunit (SU), calmodulin, Troponin, Importin beta, Fte-1, estrogen receptor-related protein (hERR1), Imogen-38, Atrophin-1, GBDR1, DGS-1, norbin (KIAA0607), OPA containing protein, and M4 protein, and ten novel LYST Interacting Proteins ("LIP-1" through "LIP-10") further characterized herein. The invention also relates to complexes of LYST-2 protein with one or more proteins chosen from the list comprising: 14-3-3 protein, XAP-4, and HBF-G2. The invention includes antibodies specific to LYST complexes and LYST-2 complexes, and their use in, *inter alia*, screening, diagnosis, prognosis and therapy. The invention further relates to novel LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5. LIP6, LIP7, LIP8, LIP9, and LIP10 nucleic acids, proteins and derivatives, fragments and analogs thereof, and antibodies specific to said novel LYST-2 protein and novel set of LIP proteins.

#### **BACKGROUND OF THE INVENTION**

#### ROLE OF LYST IN DISORDERS AND DISEASES

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The lysosomal Chediak-Higashi syndrome protein LYST (GenBank Accession No. U67615), is the human homolog of the mouse lysosomal trafficking regulator beige (BG). (Barbosa et al., 1996, Nature 382: 262-265; Barbosa et al., 1997, Human Mol. Genetics 6: 1091-1098). Both proteins are crucial for lysosomal, and hence cellular, function. The identification of interactions between LYST and other proteins would be useful in the development of treatments and assays for atopic disorders related to malfunctions or deficiencies in lysosomes

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and lysosome-related disorders and diseases. Examples of disorders and disease states relevant to this invention are classified below.

#### CHEDIAK-HIGASHI (CH) SYNDROME

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Chediak-Higashi syndrome (CHS) is an autosomal recessive immune deficiency disease found in humans and modeled in "beige" (bg) mouse strains. Affected individuals have giant, perinuclear lysosomes, and defective granulocytic, natural killer and cytolytic T cell function. The disorder is lethal in that the affected individuals die prematurely of infection or malignancy (Baetz et al., 1995, J. Immunol. 154: 6122-6131). The disorder is characterized by hypopigmentation, severe immunologic deficiency with neutropenia, lack of natural killer cells, abnormal platelet function (bleeding tendency), and neurologic disorders such as peripheral neuropathy and ataxia (Chediak, 1952, Rev. Hematol 7: 362-367; Higashi, 1954, Tochoku J. Exp. Med. 59: 315-332).

Intracellular protein transport to and from lysosomes is disordered in CHS patients; giant vesicles arise from lysosomes and proteins are mis-sorted into cell compartments. Functional defects in the secretory lysosomes of granular cells (leukocytes, melanocytes, megakaryocytes and cerebellar Purkinje cells) provide a unifying hypothesis that explains the diverse clinical features of CHS (Griffiths, 1996, Semin. Immunol. 9: 109-115). Impaired cytolysis by natural killer cells and cytotoxic T cells contribute to the immunodeficiency of CHS and is probably the result of indolent exocytosis of giant lytic granules upon target cell binding (Baetz et al., 1995, J. Immunol. 154: 6122-6131). Melanosomal tyrosine hydroxylase from melanocytes (Windhorst et al., 1968, J. Invest. Dermatol. 50: 9-18) and platelet dense granule constituents (Novak et al., 1985, Blood 66: 1196-1201) are mis-sorted in CHS which result in other features such as albinism, bleeding diathesis and neurodegenerative disease. Albinism reflects a deficiency of tyrosinase in melanocytes due to exocytosis instead of delivery to the melanosome. Impaired platelet aggregation and a bleeding tendency are due to absence of dense granules in megakaryocytes and platelets.

Along with protein sorting defects, giant inclusion bodies and giant intracellular vesicles, including lysosomes, melanosomes, platelet dense granules and cytolytic granules, are formed in CHS patients (Perou et al., 1997, J. Biol. Chem. 272: 29790-29794, Jones et al., 1992, Clin. Immunol. Immunopath. 65: 219-226; Burkhardt et al., 1993, J. Exp. Med. 178: 1845-1856; Holchombe et al., 1994. Immunodeficiency 5: 131-140). Similar abnormalities occur in the beige (bg) mouse (Lutzner et al., 1966, Heredity 58: 299-300). Vesicular transport to and from the

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lysosome and late endosome is defective along with aberrant compartmentalization of lysosomal and granular enzymes in both humans with CHS and in mice carrying the *beige* mutation. A role of beige/CHS protein as a component of a membrane-associated signal transduction complex that regulates intracellular protein trafficking has been inferred from cellular studies. Phenotypic studies suggest that the primary defect in CHS is a mutation in a protein which regulates the budding, docking, or fusion of lysosomal and target membranes.

The mouse beige (BG) protein and the human Chediak-Higashi proteins (LYST) have been cloned and have been shown to be homologous (Barbosa et al., 1996, Nature 382: 262-265; Barbosa et al., 1997, Human Mol. Genetics 6: 1091-1098). The 11,403 bp open reading frame of the LYST gene predicts a polypeptide of 3,801 amino acids with a molecular mass of 429,153 Da (Nagle et al., 1996, Nature Genetics 14: 307-311). Said homologs have a conserved domain called BEACH (beige and CHS). LYST protein is predicted to form helical domains (Barbosa et al., 1996, Nature 382: 262-265) and consists of a series of hydrophobic helices that most closely resemble ARM (Peifer et al., 1994, Cell 76: 789-791) and HEAT (Andrade & Bork, 1995, Nature Genet. 11: 115-116) repeat motifs which tend to form long rods. Many of the known HEAT repeat motif-containing proteins are associated with vesicle transport.

The C-terminal region of LYST contains seven consecutive 'WD' repeat motifs (Sondek et al., 1996, Nature 379: 369-374), which form beta sheets arranged in a 7-bladed propeller-like secondary structure. This region is similar to the beta-subunit of heterotrimeric G proteins and is thought to mediate protein-protein interactions. LYST has a region of sequence similar to stathmin (oncoprotein 18), a coiled-coil phosphoprotein that regulates microtubule polymerization and acts as a relay for intracellular signal transduction (Barbosa et al., 1996, Nature 382: 262-265).

The LYST transcript predominates in thymus, fetal thymus, spleen and brain. LYST is abundant in brain tissues, peripheral blood leukocytes and bone marrow. The expression distribution of mRNA isoforms in humans is consistent with the pattern of clinical features observed in CHS patients (immune deficiency, platelet storage pool deficiency, neurologic manifestations, albinism) (Barbosa et al., 1997, Human Mol. Genetics 6: 1091-1098).

In summary, LYST is centrally implicated in many functional activities including, but not limited to, physiological processes such as signal transduction, vesicular transport, the formation and exocytosis of acidic intracellular organelles, and protein trafficking; pathological processes, including but not limited to, atopic diseases, autoimmune diseases, degenerative disorders such

as neurodegenerative disease, hyperproliferative disorders such as tumorigenesis and tumor progression, oculocutaneous albinism, hypopigmentation, and platelet dysfunction.

#### **DEFICIENCIES IN THE PRIOR ART**

Some methods for the treatment and diagnosis of Chediak-Higashi Syndrome have been suggested on the basis of the isolation and characterization of the CH gene in mice (beige) and humans (LYST) (International Patent WO 97/28262). Despite these recent studies, there is only speculation that the LYST protein interacts with other proteins in the cell. Nothing is known about which proteins actually interact with LYST. In mice, it has been suggested that the cytoplasmic beige protein may associate with intermediary proteins that in turn interact with the intracellular membranes of vesicles and organelles. A protein interaction domain in LYST is suggested by the presence of the 'WD' repeats, which have been shown to be a protein-protein interaction motif.

In addition to contributing to an understanding of lysosomal trafficking, identification of LYST-interacting proteins is of medical significance for several reasons:

- (1) Firstly, improved molecular understanding of Chediak-Higashi Syndrome (CHS) may suggest treatments that are not currently being considered. Patients with CHS still typically die in childhood.
- (2) Secondly, novel therapeutic targets may be identified for control of mast cell degranulation and amelioration of consequent damage in diseases such as asthma and urticaria.
- (3) Thirdly, the observation that CHS mice are protected from lupus nephritis (Clark et al., 1982) suggests that the LYST pathway represents a therapeutic target in autoimmune disease.

By using a modified yeast two hybrid system, this invention identifies a rather large number of LYST interacting proteins. The identification of interactions between LYST and other proteins can be useful in the development of treatments and assays for atopic disorders such as asthma, nasal polyps, hay fever rhinitis, and urticaria; autoimmune diseases such as CHS, rheumatoid arthritis, systemic lupus, inflammatory bowel disease, diabetes mellitus, and multiple sclerosis; neurological disorders and diseases; certain forms of cancer; pigmentation defects; and bleeding tendency.

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#### SIGNALING PROCESSES AND PROTEIN TRAFFICKING

The following section describes previously identified proteins, herein identified as interacting with LYST, which are involved in signaling processes and protein trafficking.

#### 5 14-3-3 PROTEIN

A LYST interactant identified in the present invention is human 14-3-3 protein (GenBank Accession No. X56468. Nielsen, 1991, *Biochim. Biophys. Acta 1088*: 425-428). A broad range of organisms and tissues contain a family of highly conserved 14-3-3 proteins, which has been associated with many diverse functions, including signal transduction, exocytosis and cell cycle regulation. One human 14-3-3 protein is an abundant acidic brain protein of approximately 28 kDa (GenBank Accession No. X56468). This protein activates tyrosine and calcium/calmodulin-dependent protein kinase II.

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14-3-3 proteins exist in cells both as membrane-associated and cytoplasmic forms (Martin et al., 1994, J. Neurochem. 63:2259-2265; Roth et al., 1994, Biochem. J. 301:305-310). They stimulate calcium-dependent exocytosis in several cell types, acting apparently at the priming stage in a manner that is potentiated by protein kinase C (PKC) and associated with actin reorganization (Chamberlain et al., 1995, J. Cell. Biol. 130:1063-1070; Burgoyne et al., 1993, J. Anat. 183:309-314; Roth and Burgoyne, 1995, FEBS Letters 374: 77-81), although, as with LYST protein, the precise mechanism is unknown.

14-3-3 proteins are also important in signal transduction: 14-3-3 protein bind PKC, is phosphorylated by PKC, and inhibits PKC activity. The PKC abnormalities described in CHS are similar to the effects of 14-3-3 on PKC (Ito et al., 1989, Biochem. Biophys. Res. Commun. 160:433-440; Sato et al., 1990, J. Leukocy. Biol. 48:377-381). 14-3-3 protein also binds to serine/threonine protein kinase Raf-1, and is necessary for Raf-1 kinase activity (1 (Zhang et al., 1997, J. Biol. Chem. 272: 13717-13724; Michaud et al., 1995, Mol. Cell. Biol. 15: 3390-3397). 14-3-3 also interacts with other kinases, such as Bcr. and can form ternary complexes with Raf and Bcr (Braselman and McCormick, 1995, EMBO J. 14: 4839-4848). Like the proposed action of LYST, 14-3-3 appears to function as a scaffold protein that facilitates interactions among components of signaling and exocytic pathways (Aitken et al., 1995, Biochem. Soc. Trans. 23:605-611).

The Raf/MECK/ERK/MAP signal transduction pathway and 14-3-3 protein have been suggested to play a role in the initiation of apoptosis. Disorders of the regulation of apoptosis may play an important role in the pathogenesis of autoimmune and atopic diseases, including bronchial asthma. AIDS, and neoplastic diseases (Jagiello & Krasnowska, 1997, *Postepy. Hig. Med. Dosw. 51*: 385-398). There is a linkage between the induction of apoptosis and signal transduction disorders.

#### 6 HS1 PROTEIN

Another member of the highly conserved and ubiquitously expressed eukaryotic 14-3-3 family of proteins is human Hs-1. also called 14-3-3 beta protein. which is an activator of tyrosine and tryptophan hydroxylases (GenBank Accession No. X57346: Leffers et al., 1993, J. Mol. Biol. 231: 982-998). HS1 protein. 80% identical to 14-3-3 protein. is related to the protein kinase C inhibitory protein (KCIP) and is an abundant and ubiquitously expressed acidic protein of approximately 28 kDa. HS1 protein is present in purified synaptic membranes. Since it is selectively expressed in synaptic membranes, HS1 protein may influence neurotransmission by regulating exocytosis (Martin et al., 1994, J. Neurochemistry 63: 2259-2265). HS1 protein also exists in phosphorylated forms in mammalian and avian brain. The HS1 protein interacts with and activates Raf-1, a mediator of mitogenesis and differentiation and a key protein involved in transmission of developmental and proliferative signals generated by receptor and nonreceptor tyrosine kinases (Aitken et al., 1995, J. Biol. Chem. 270: 5706-5709; Fantl et al., 1994, Nature 371: 612-614). HS1 protein also interacts with insulin receptor substrate-1 (Kosaki et al., 1998, J. Biol. Chem. 273: 940-944) (see Imogen 38, infra).

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# HEPATOCYTE GROWTH FACTOR-REGULATED TYROSINE KINASE SUBSTRATE PROTEIN (HRS)

Hepatocyte growth factor-regulated tyrosine kinase substrate ("Hrs") protein (GenBank Accession No. D84064, see, e.g., Lu, et al., 1998, Gene 213: 125-135) has been shown in the present invention to interact with LYST. The region of Hrs that interacts with LYST (amino acids 84-777) contains a structurally conserved putative zinc finger domain and an ATPase catalytic site. Hrs is localized in the cytoplasm of the cell. Hrs is 80% identical to rat Hrs-2, which is an ATPase.

Importantly, Hrs has two function that may also explain the mechanism of action of LYST. Firstly, Hrs inhibits exocytosis in a dose-dependent manner, probably through binding to SNAP-25, a component of the SNARE protein complex ((Bean et al., 1997, Nature 385: 826-829).). While the SNARE complex has a well-defined role in synaptic vesicle docking, there is also evidence for SNAP25 involvement in calcium dependent exocytic membrane fusion reactions (Banerjee et al., 1996, J. Biol. Chem. 271:20227-20230). Calcium and zinc inhibit binding of Hrs to SNAP25 and promote exocytosis (Bean et al., 1997, Nature 385: 826-829)

Secondly, Hrs interacts with STAM (signal transducing adaptor molecule), a relay in cytokine-mediated intracellular signal transduction (Asao et al., 1997. J. Biol. Chem. 272:32785-

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32791). In binding to STAM. Hrs suppresses STAM-mediatd signaling. Cytokine stimulation of cells leads to STAM-mediated cell growth signalling and phosphorylation of Hrs.

#### **BMK1 ALPHA KINASE**

Another LYST interactant identified in the present invention is BMK1 alpha kinase (GenBank Accession Number U29725). The mitogen-activated protein (MAP) kinase BMK1 is part of a distinct MAP-kinase signaling pathway that is required for EGF-induced cell proliferation and progression through the cell cycle. see. e.g., Lee et al., 1995, Biochem. Biophys. Res. Commun. 213: 715-724. A redox-sensitive kinase, BMK1 plays an important role in shear stress-mediated gene expression in endothelial cells.

#### **KB07**

Another LYST interactant identified in the present invention is KB07 kinase (GenBank Accession Number AF064606, direct submission). The gene was isolated from human dendritic cells and is similar to tyrosine kinase. Thus, it is likely that KB07 plays a role in the signaling pathway.

#### **EFS**

Another of the LYST interactants identified in this invention is the human phosphoprotein signaling protein Efs (embryonal Fyn-associated substrate) (GenBank Accession No. AB001466; Ishino et al., 1997, Oncogene 15: 1741-1745). Efs has characteristic regions important in intracellular signal transduction. including a Src homology 3 (SH3) domain, a cluster of putative ligands for Src homology 2 (SH2) domains, and proline-rich sequences with SH3-binding consensus (Ishino et al., 1995, Oncogene 11: 2331-2338). Efs has been found to be associated with Fyn, a nonreceptor protein tyrosine kinase that plays a role in signaling processes, including T-cell activation, excitation-contraction coupling, and programmed cell death (apoptosis) (Marks, 1997, Am. J. Physiol. 272: H597-H605).

#### **OS9 PRECURSOR**

Another one of the LYST interactants identified in this invention is OS-9 (GenBank Accession No. U41635). The gene encoding the OS-9 precursor is located within chromosome 12q13-15, a region frequently amplified in human cancers. In addition, the gene is ubiquitously expressed in human tissues and amplified in sarcomas, frequently coamplified with the CDK4

gene. See, e.g. Su et al., 1996. Mol. Carcinog. 15: 270-275. Thus, it may be involved in cellular growth control.

## CASEIN-KINASE II BETA SUBUNIT

Another LYST interactant identified in the present invention is the ubiquitous serine/threonine protein kinase, casein-kinase II beta subunit (casein kinase II beta SU) (GenBank Accession Number M30448, Jakobi et al., 1989, Eur. J. Biochem. 183: 227-233). The highly conserved enzyme is present in the nucleus and cytoplasm, and is another LYST interactant with roles in both signal transduction and the regulation of vesicular trafficking.

Casein kinase II beta SU is a regulatory subunit that stabilizes the catalytic alpha-subunit and modulates interactions with substrates (Allende & Allende, 1995, FASEB J. 9: 313-323). Several casein kinase II beta SU substrates are important in vesicular transport, including synaptotagmin, syntaxin (a t-SNARE), synaptobrevin (a v-SNARE), calmodulin, protein kinase C, UNC18 and clathrin (Daveltov et al., 1993, J. Biol. Chem. 268:6816-6822; Nielander et al., 1995, J. Neurochem. 65:1712-20; Sacks & Mazus, 1994, Biochem. Mol. Biol. Int 34:251-259; Foster et al., 1998, Biochemistry 37:11089-96; Shimanzki et al., 1996, J. Biol. Chem. 271:14548-53).

#### CALMODULIN

Another LYST interactant identified in the present invention is calmodulin (GenBank Accession Number D45887), a low molecular weight cytoplasmic protein that is basically involved in many calcium-dependent interactions, including exocytosis. The twelve carboxy-terminal amino acids of calmodulin interact with the N-terminus of LYST, as disclosed in this invention. These carboxy-terminal amino acids form the end of a calcium-binding site and an alpha-helix.

Calmodulin has a significant role in membrane fusion: it binds to intracellular organelles such as secretory granules, and is required for membrane fusion and exocytosis in several cell types that are aberrant in CHS, including platelets, mast cells and leukocytes. (Sheng *et al.*, 1996, *Nature 379*: 4541-454) In particular, SNARE-mediated docking of acidic organelles has been shown to prompt local calcium release, causing calmodulin to bind tightly to the docked vesicle and promote membrane fusion (Peters & Mayer, 1998, Nature 396:575-580; Schekman, 1998, Nature 396:514-515).

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Furthermore, calmodulin inhibits interaction of Hrs and SNAP25 (see above). The Ca2+-signaled exocytosis is blocked by SNAP-25, a protein of the vesicle docking complex (see background on Hrs, *supra*). Alpha-SNAP, 14-3-3 proteins, and calmodulin effect regulation of exocytosis in permeabilized adrenal chromaffin cells (Chamberlain *et al.*, 1995, *J. Cell Biol. 130*: 1063-1070). All three proteins, alpha-SNAP, 14-3-3 and calmodulin, lead to a Ca2+-dependent increase in catecholamine secretion. These three proteins also have distinct stage-specific actions on exocytosis.

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#### TROPONIN I

Another LYST interactant identified in the present invention is the troponin I (GenBank Accession Number X54163), a protein involved in muscle filament contraction. Troponin I is a subunit of the thin filament-associated troponin-tropomyosin complex involved in calcium regulation of skeletal and cardiac muscle contraction. Cytoskeletal proteins including Troponin I play a central role in cardiomyopathies. Cardiomyopathies are serious heart muscle disorders in children and adults, which result in morbidity and premature death.

#### **IMPORTIN BETA SUBUNIT**

Another LYST interactant in the present invention was identified as the Importin beta subunit (GenBank Accession No. L38951, Gorlich et al., 1995, Curr. Biol. 5: 383-392). Importin. a soluble protein consisting of a 60 kDa (alpha) and 90 kDa (beta) subunit. is essential for the first step of selective protein import into the cell nucleus. The complex of Importin-beta and Importin-alpha is essential for nuclear protein import. Importin-alpha provides the nuclear localization signal binding site, Importin-beta the site of initial docking to the pore (Gorlich et al, 1995, Nature 377: 246-248). Importin-beta interacts with Rna1p, a GTPase activating protein required for nuclear transport (Koepp et al., 1996, J. Cell Biol. 133: 1163-1176).

The human immunodeficiency virus type 1 (HIV-1) Rev protein nuclear localization signal mediates specific binding to human Importin-beta (Henderson & Percipalle, 1997, *J. Mol. Biol. 274*: 693-707). The Rev protein binds to unspliced HIV-1 pre-mRNA and exports it from the nucleus. Rev itself can "shuttle" between the nucleus and cytoplasm. This bi-directional transport is mediated by two specific Rev sequences: a nuclear localization signal and a distinct nuclear export signal. Rev and Importin-alpha bind (via an arginine-rich sequence) to a similar region on Importin-beta. Thus, the Importin-beta subunit is an essential component of the nuclear protein translocation complex.

10 FTE-1

The fie-1 gene. a putative v-fos transformation effector gene (GenBank Accession Number M84711; Kho & Zarbl. 1992, Proc. Natl. Acad. Sci. USA, 89: 2200-2204), has been shown to encode ribosomal protein S3a. An elevated expression of fte-1 mRNA is frequently associated with cell transformation. As a result, the accumulation of ribosomal subunits modulates neoplastic transformation and cell growth. In addition to the role in cell transformation, fte-1 might be involved in protein translocation into mitochondria, due to its homology to a yeast gene (MFT1), whose product is involved in protein import.

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### ESTROGEN RECEPTOR RELATED PROTEIN (hERRal)

The human estrogen receptor-related protein (hERRa1) was identified in the present invention as one of the LYST interactants. hERRa1 (GenBank Accession Number X51416, Yang et al., 1996, J. Biol. Chem. 271: 5795-5804) is an orphan member of the steroid-thyroid hormone receptor superfamily of transcription factors which are expressed in characteristic patterns during organogenesis and postnatal development. hERRa1 plays a role in bone development and metabolism, and was found expressed in osteoblastic osteosarcoma cell lines (Bonnelye et al., 1997, Mol. Endocrinol. 11: 905-916). hERRa1 interacts with the human estrogen receptor.

Steroid hormone receptors are basically localized in the nucleus, regardless of hormonal status, and considerable amounts of unliganded steroid hormone receptors may be present in the cytoplasm of target cells in exceptional cases (Yamashita, 1998, *Histol. Histopathol. 13*: 255-270). Estrogens transiently induce a number of nuclear protooncogenes, such as members of the c-fos and c-jun family of proteins, which act as transcription factors through the estrogen receptor system. In addition, the estrogen receptor system appears to participate in the growth response and abnormalities of the epithelium elicited by exogenous estrogen treatment during the neonatal period (Yamashita, 1998, *Histol. Histopathol. 13*: 255-270).

#### **IMOGEN 38**

The human Imogen 38 protein (GenBank Accession Number Z68747) was identified in this invention as one of the LYST interactants, and is a novel 38 kDa islet mitochondrial autoantigen (Arden et al., 1996, J. Clin. Invest. 97: 551-561). Cell-mediated autoimmune attack directed against Imogen 38 is associated with type 1 diabetes. Insulin regulates glucose uptake, mediated by an translocation of glucose transporters from an intercellular vesicular pool to the

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plasma membrane. This process is similar to the regulated docking and fusion of vesicles in neuroendocrine cells (Cheatham et al., 1996. Proc. Natl. Acad. Sci. U.S.A. 93: 15169-15173).

#### XAP-4

The protein encoded by the XAP-4 gene was found to be a LYST-2 interacting protein. The XAP-4 mRNA (GenBank Accession Number X79353) encodes for a rap GDP-dissociation inhibitor. See, e.g., Sedlacek et al., 1994. Mamm. Genome 5: 633-639. Thus, it may play a role in regulating the GDP-GTP exchange reaction, and may therefore be involved in signaling processes.

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# PROTEINS INVOLVED IN NEURODEGENERATIVE AND DEVELOPMENTAL DISORDERS

The following section describes previously identified proteins, herein identified as interacting with LYST, which are involved in neurodegenerative and developmental disorders.

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#### ATROPHIN-I

Another LYST interactant identified in the present invention is Atrophin I (GenBank Accession Number U23851, Margolis et al., 1996, Brain Res. Mol. Brain Res. 36: 219-226). Atrophin-I is the protein product of the gene for dentatorubral pallidoluysian atrophy (DRPLA, Smith's disease). DRPLA is a rare, progressive, fatal autosomal dominant neurological disorder characterized by neuronal degeneration, especially in the cerebellar dentate nucleus. Clinical symptoms include variable combinations of myoclonus epilepsy, cerebellar ataxia, choreoathetosis and dementia. DRPLA is known to result from expansion of a CAG trinucleotide repeat encoding glutamine (Margolis et al., 1996, Brain Res. Mol. Brain Res. 36: 219-226).

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#### **GBDR1**

A further LYST interactant described in this invention is the putative glialblastoma cell differentiation-related protein (GBDR1) (GenBank Accession Number AF068195, direct submission).

# DIGEORGE SYNDROME PROTEIN (DGS-I)

An additional LYST interactant described in this invention is the DiGeorge syndrome (DGS)-I protein (GenBank Accession Number L77566; Gong et al., 1996, Hum. Mol. Genet. 5: 789-800). The DGSI gene is located within the minimal DiGeorge syndrome critical region of the chromosome and encodes for a 476 amino acid protein (Gong et al., 1997, Hum. Mol. Genet. 6: 267-276). Patients with DiGeorge syndrome have deletions of chromosomal region 22q11.2. The clinical symptoms include cardiac defects, thymic hypoplasia or aplasia and hypocalcemia.

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#### **NORBIN (KIAA0607 GENE)**

One LYST interactant described in this invention is the human homolog to the rat norbin gene (GenBank Accession Numbers AB011179 for human KIAA0607 gene and AB006461 for rat norbin; Shinozaki et al., 1997, Biochem. Biophys. Res. Comm. 240: 766-771) Rat norbin is expressed in the brain and is induced by treatment of tetraethylammonium in rat hippocampal slice accompanied with neurite-outgrowth in neuro-2a cells. The neurite-outgrowth-related norbin protein may play a role in neural plasticity because of the formation of new synapses.

#### **OPA CONTAINING PROTEIN**

A LYST interactant was identified as an OPA-containing protein (GenBank Accession No. AF071309, Philbert, et al., 1998, Mol. Psych. 3: 303-309). No function has yet been assigned to this protein.

#### **M4 PROTEIN**

One LYST interactant was found to be identical to the M4 protein (GenBank Accession No. L03532, Datar, et al., 1993, Nucl. Acids Res. 21: 439-446). The M4 protein is a RNA-binding protein.

#### **HUMAN BRAIN FACTOR (HBF)-G2**

A protein that was found to interact with LYST-2 in the present invention was identified as human brain factor (HBF)-G2 (GenBank Accession Number X78202; Wiese et al., 1995, Biochim. Biophys. Acta 1262: 105-112). HBF-G2 is a member of the fork head gene family, which expression is restricted to the neurons of the developing telencephalon. HBF-G2 is strongly expressed in embryonic brain and may contribute to early developmental decisions in cell fates during embryogenesis.

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It should be noted that the citation of a reference in this or in any section of the specification shall not be construed as an admission that such reference is prior art to the present invention.

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#### SUMMARY OF THE INVENTION

The human Chediak-Higashi syndrome protein LYST plays an important role in regulating lysosomal trafficking and function. The inventors have cloned a large novel human gene fragment, hereforth referred to as LYST-2, whose DNA coding region and translated protein sequence are highly homologous to human LYST. The LYST-2 protein contains a BEACH domain. The present inventors suggest that LYST-2 also plays an important role in lysosomal regulation. The invention herein provides a composition comprising the *LYST-2* gene or protein, as well as derivatives, fragments, and analogs thereof.

The present invention is based upon the inventors' discovery that certain proteins bind to and form complexes with LYST or LYST-2 proteins, as assayed by a modified yeast two-hybrid system (Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88: 9578-9581). Accordingly, the invention also provides compositions comprising a protein complex of a LYST or LYST-2 protein with a protein that interacts with, i.e., binds to, LYST or LYST-2. A protein shown to bind with LYST or LYST-2 is designated as a "LYST-IP" for LYST-Interacting Protein, and the complexes of LYST or LYST-2 and a LYST-IP are designated as "LYST:LYST-IP" herein. Specifically, the present invention is directed to complexes of LYST or LYST-2, and complexes of derivatives, fragments and analogs of LYST or LYST-2, with 14-3-3 protein, with HS1 protein, with Hrs, with BMK1, with KB07, with Efs, with XAP-4, with OS9, with casein kinase II beta SU, with calmodulin, with troponin I, with Importin beta, with Fte-1, with estrogenreceptor related protein, with Imogen 38, with Atrophin-1, with norbin, with HBF-G2, with DGS-I, with GBDR-1, with OPA containing protein, and with M4 protein and their derivatives. analogs and fragments of these LYST or LYST-2 interacting proteins. Ten genes that interact with LYST or LYST-2 are novel to this invention, and are identified herein as LIP-1, LIP-2, LIP-3. LIP-4. LIP-5. LIP-6, LIP-7. LIP-8. LIP-9. and LIP-10. These ten novel genes have not been previously described, and translation products of mRNAs of these genes have not been previously defined. Therefore, the present invention is also directed to complexes of LYST or LYST-2, and complexes of derivatives, fragments and analogs of LYST or LYST-2, with LIP1,

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with LIP2. with LIP3. with LIP4. with LIP5. with LIP6, with LIP7, with LIP8. with LIP9, and with LIP10 and their derivatives, analogs and fragments of these LYST or LYST-2 interacting proteins. Antibodies specific to complexes of LYST or LYST-2 and at least one LYST-IP, and antibodies specific to complexes of derivatives, fragments or analogs of said LYST or LYST-2 and at least one LYST-IP, wherein any one or more of the LYST, LYST-2, and LYST-IP proteins may be a derivative, analog or fragment thereof.

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The invention further relates to nucleotide and amino acid sequences of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10 (the human LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10 and homologs of other species), as well as derivatives, fragments, and analogs thereof. Nucleic acids able to hybridize to, or complementary to, the foregoing nucleotide sequence, such as the inverse complement of the foregoing sequence, are also provided. (The inverse complement is a nucleic acid sequence that has the complementary sequence running in reverse orientation to the strand so that the inverse complement would hybridize without mismatches to the nucleic acid strand.)

The invention also relates to protein derivatives and analogs of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 that are functionally active. *i.e.*, they are capable of displaying one or more known functional activities of said wild-type proteins. Such functional activities include, but are not limited to, (i) the ability to bind with or compete for interaction with LYST; (ii) antigenicity, *i.e.*, the ability to bind or compete with LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 for binding to each LIP-specific antibody, respectively; and (iii) immunogenicity, *i.e.*, the ability to generate an antibody that binds LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, respectively.

Methods of production of the LYST:LYST-IP complex and of the LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, and derivatives, fragments, and analogs of the complexes and/or individual proteins, *e.g.*, by recombinant means, are also provided. Pharmaceutical compositions comprising same are also provided.

The present invention further provides methods of modulating (i.e., inhibiting or enhancing) the activity of LYST:LYST-IP complexes, and methods of modulating a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein. The individual protein components of the above mentioned LYST:LYST-IP complexes have been implicated in cellular functions, including but not limited to, (i) physiological processes including, but not restricted to, signal transduction, vesicular transport, the formation and exocytosis of acidic, intracellular

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organelles, protein trafficking, pigmentation regulation, platelet function, and viral response; and (ii) pathological processes including, but not restricted to, atopic diseases, autoimmune diseases, degenerative disorders including neurodegenerative disease, hyperproliferative disorders including tumorigenesis and tumor progression, oculocutaneous albinism, hypopigmentation, platelet dysfunction and viral infections.

Accordingly, the present invention also provides methods for screening a LYST:LYST-IP complex, and screening a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, as well as derivatives and analogs of the LYST:LYST-IP complex; screening a LYST-2, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 mRNA; and screening a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein for the ability to alter cell functions, particularly those cell functions in which LYST, LYST-2, and/or a LYST-IP have been implicated.

The present invention also relates to therapeutic and prophylactic, as well as diagnostic, prognostic, and screening methods and compositions based upon LYST:LYST-IP complexes (and the nucleic acid molecules encoding the individual proteins that participate in the complexes), as well as LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 or LIP10 proteins and encoding nucleic acid molecules. Therapeutic compounds of the invention include, but are not limited to, LYST:LYST-IP complexes and complexes where one or both members of the complex is a derivative or analog of LYST, LYST-2, and/or a LYST-IP. Therapeutic compounds also include LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins and derivatives, fragments or analogs thereof, antibodies to the protein or derivatives, fragments or analogs of the antibody, and nucleic acids encoding the foregoing proteins, derivatives. fragments and analogs. Therapeutic compounds also include antisense nucleic acids to the nucleotide sequences encoding the LYST:LYST-IP complex components, and LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 antisense nucleic acids. Diagnostic, prognostic and screening kits are also provided.

Animal models and methods for screening for modulators (i.e., agonists, antagonists and inhibitors) of the activity of a LYST:LYST-IP complex, or of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, are also provided in the present invention.

Methods for identifying molecules that inhibit, or alternatively, that increase formation of LYST:LYST-IP complexes are also provided in the present invention.

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# BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1: is a depiction of the novel nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of LYST-2. The coding sequences beginning at base 3. indicated by an arrow, and ending at base 794, and beginning at base 774, indicated by arrow, and ending at base 1424 were used as "bait" in the assays described in the EXAMPLES, infra.

FIGURE 2: is a depiction of the LIP-1 nucleotide sequence (503 nucleotides total, SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4). LIP-1 is a TCP10A homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at nucleotide 1 and 5. indicated by arrow. The known sequence does not include the initiation codon, and therefore, the Ile residue at amino acid position 1 is denoted in bold indicating that the sequence must be extended in the amino-terminal direction. The sequence does not have a STOP codon. The amino acid at position 85 (numbered from the position 1 Ile residue) may be encoded by AAC (Asn), ACC (Thr), AGC (Ser) or ATC (Ile).

FIGURE 3: is a depiction of the LIP-2 nucleotide sequence (495 nucleotides total, GenBank Accession No. AA010799) (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6). The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at bases 1, 10, and 21.

FIGURE 4: is a depiction of the LIP-3 nucleotide sequence (1198 residues, SEQ ID NO:7) and amino acid sequence (SEQ ID NO:8, respectively). LIP-3 is a ROAZ homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at base 345.

FIGURE 5: is a depiction of the LIP-4 nucleotide sequence (total of 524 nucleotides, SEQ ID NO:9) and amino acid sequence (SEQ ID NO:10). LIP-4 is a hnRNP-E2 homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at base 2. As the protein translation depicted does not begin with an "ATG" initiator methionine codon, we surmise the protein sequence encoded by nucleotide residues 71 to 898, as shown, represents a C-terminal protein fragment, wherein the N-terminal protein sequence is not represented in the figure.

FIGURE 6: is a depiction of the LIP-5 nucleotide sequence (total of 517 nucleotides, SEQ ID NO:11) and amino acid sequence (SEQ ID NO:12). The prey sequence identified in the assay described in the Examples. *infra*. begins at base 48.

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FIGURE 7: is a depiction of the LIP-6 nucleotide sequence (total of 753 nucleotides. SEQ ID NO:13) and amino acid sequence (SEQ ID NO:14). LIP-6 is an ns2-3 homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at base 1.

FIGURE 8: is a depiction of the LIP-7 nucleotide sequence (total of 451 nucleotides. SEQ ID NO:15) and amino acid sequence (SEQ ID NO:16). LIP-7 is a TCP10A homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at base 1. indicated by an arrow.

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FIGURE 9: is a depiction of the LIP-8 nucleotide sequence (total of 402 nucleotides, SEQ ID NO:17) and amino acid sequence (SEQ ID NO:18). LIP-8 is a KAP4L homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at base 1, indicated by an arrow.

FIGURE 10: is a depiction of the LIP-9 nucleotide sequence (total of 554 nucleotides, SEQ ID NO:19) and amino acid sequence (SEQ ID NO:20). LIP-9 is an etr-1 homolog. The prey sequence identified in the assay described in the EXAMPLES, *infra*, begins at base 138, indicated by an arrow. As the protein translation depicted does not begin with an "ATG" initiator methionine codon, we surmise the protein sequence encoded by nucleotide residues 78 to 716, as shown, represents a C-terminal protein fragment, wherein the N-terminal protein sequence is not represented in the figure.

FIGURE 11: is a depiction of the LIP-10 nucleotide sequence (total of 373 nucleotides, SEQ ID NO:21) and amino acid sequence (SEQ ID NO:22). LIP-10 is an chicken tyrosine kinase homolog. The prey sequence identified in the assay described in EXAMPLES. *infra*. begins at base 1.

FIGURE 12: demonstrates the specificity of LYST interactions. Shown is the matrix of results of the yeast two hybrid system assays. The results of assays using the bait proteins LYST and LYST-2 are indicated above the columns. LYST was used in two screens, a forward (LYST FOR) and a reverse (LYST REV) screen. The prey proteins 14-3-3 protein, HS1 protein, Hrs. BMK1, KB07. Efs. XAP-4, OS9, casein kinase II beta SU. calmodulin, troponin I, importin beta. Fte-1, ERRa1. imogen 38, atrophin-1, norbin, HBF-G2. DGS-I, GBDR1, OPA containing protein, M4 protein. LIP1, LIP2, LIP3, LIP4, LIP5. LIP6, LIP7, LIP8, LIP9, and LIP10. CDK2. retinoblastoma. p27(Kip1), RGL-2 and vector control are indicated to the left of the columns. A positive interaction between the indicated bait and prey proteins is indicated as "+" in the box forming the intersection between the particular bait and prey proteins. a lack of interaction is designated by "-". N.D. = not determined in this test.

FIGURE 13: is a depiction of the corrected nucleotide sequence (SEQ ID NO:23) and deduced amino acid sequence (SEQ ID NO:24) of LYST-2. The coding sequences beginning at base 3, indicated by an arrow, and ending at base 794, and beginning at base 774, indicated by arrow, and ending at base 1424 were used as "bait" in the assays described in the EXAMPLES, infra.

# DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon two proteins important in regulating lysosomal trafficking and function, namely, LYST and a novel LYST homolog described herein and termed LYST-2. LYST-2 has a 74% amino acid similarity to LYST, and its protein contains the conserved BEACH domain described in the Chediak-Higashi LYST protein and the murine beige protein. Thus, one embodiment of the present invention provides a composition comprising the *LYST-2* gene or LYST-2 protein [SEQ ID NO:1 and SEQ ID NO:2, respectively (FIG. 1) and the more complete SEQ ID NO:24 and SEQ ID NO:24 (FIG. 13)], and derivatives, fragments, and analogs thereof.

# LYST:LYST-IP COMPLEXES AND LIP PROTEINS

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The invention is directed toward the inventors' discovery that certain proteins bind to and form complexes with LYST or LYST-2 proteins (a said LYST or LYST-2 interacting protein being designated herein as a "LYST-IP" for LYST-Interacting Protein). Said LYST-IPs were identified using an improved, modified form of the yeast two hybrid system. See *e.g.* Chien, *et al.*, *Proc Natl Acad Sci USA*. 88: 9578-9581, 1991. The "LYST-IPs" 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, ERRa1, imogen 38. atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein. LYST-interacting protein-1 (LIP-1, SEQ ID NO:3), LYST-interacting protein-2 (LIP-2, SEQ ID NO:5), LYST-interacting protein-3 (LIP-3, SEQ ID NO:7), LYST-interacting protein-4 (LIP-4, SEQ ID NO:9), LYST-interacting protein-5 (LIP-5, SEQ ID NO:11). LYST-interacting protein-6 (LIP-6, SEQ ID NO:13), LYST-interacting protein-7 (LIP-7, SEQ ID NO:15), LYST-interacting protein-8 (LIP-8, SEQ ID NO:17), LYST-interacting protein-9 (LIP-9, SEQ ID NO:19), and LYST-interacting protein-10 (LIP-10, SEQ ID NO:21), were found to form complexes under physiological conditions with LYST or LYST-2 (complexes of LYST or LYST-2 with a LYST-IP are indicated as "LYST:LYST-IP" complexes

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herein). More specifically, compositions of LYST or LYST-2 complexed with one or more of the above listed proteins have not been previously described, and are novel to the present invention. These LYST:LYST-IP complexes, by virtue of the interaction, are implicated in modulating the functional activities of LYST or LYST-2 and its binding partners. Such functional activities include, but are not limited to, the physiological processes and the pathological processes that occur within individual cells, or that occur throughout the tissues and organs of an organism in question.

Hepatocyte growth factor-regualted tyrosine kinase substrate, Hrs, was found to interact with LYST fragement 6586-7449. This sequence was isolated once from a screen of a fetal brain cDNA library. The 5' end of the Hrs clone corresponds to amino acid 84.

The N-terminal region of LYST interacted with 14-3-3 protein. Four colonies, representing two independent isolates of 14-3-3 protein were identified in reverse two-hybrid screens of the fetal brain cDNA library (see Table Ib). Another LYST fragment (bp 4009-4821) interacted with the same region of 14-3-3 protein. This interaction was found once in a reverse screen of a fetal brain library (see Table Ib). In addition, eight colonies representing seven independent isolates of 14-3-3 protein were identified in forward two-hybrid screens of fetal liver, fetal brain, and adult heart libraries (see Table Ia). The LYST-interacting domain of 14-3-3 protein (amino acids 77-245) did not contain the dimerization domain, membrane-binding domain, or PKC-inhibition domain, but did include the annexin-like domain that is important in promoting Calcium-regulated exocytosis.

In addition to 14-3-3 protein, the LYST N-terminus interacted with 14-3-3 beta (also called HS1 protein) with has 82% amino acid identity to 14-3-3 protein. The HS1 interaction was identified twice in screens of a fetal brain library (Table Ib). The LYST-interacting region of HS1 protein (amino acids 31-246) was the same as the LYST-interacting region of 14-3-3 protein. Neither of the 14-3-3 interacting domains of LYST contained the recently described consensus 14-3-3 binding motif (Yaffe et al., 1997, Cell 91:961-71). Interestingly, 14-3-3 protein was also found to interact with LYST-2 fragment 774-1424 (Table Ic).

The LYST N-terminus also interacted with the C-terminal twelve amino acids of calmodulin, an interaction identified once in a screen of a fetal brain library (Table Ib). The LYST N-terminus also interacted with the C-terminal half of a steroid hormone receptor, estrogen receptor-related protein (hERR1), an interaction detected three times in a screen of a fetal brain library (Table Ib).

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The LYST fragment 2347-3213 was found to interact with the C-terminus of BMK1 alpha kinase in an interaction detected three times representing two different isolates in a reverse screen of a fetal brain library (Table Ib). The LYST fragment 3190-4032 was found to interact with the carboxy-terminal half of OS-9 precursor in an interaction detected three times in a forward screen of a keratinocyte library (Table Ia). The same LYST fragment interacted with the N-terminal part of troponin I, as detected in nine colonies representing two different isolates in a forward screen of a keratinocyte library (Table Ia). The LYST fragment 4819-5700 was found to interact with the fos transformation effector gene Fte-1 in an interaction detected twice in a forward screen of a fetal brain library (Table Ia).

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The LYST fragment 6586-7449 interacted with twelve proteins: the c-terminal halves of the importin beta-subunit, imogen 38 and norbin; DGS-I; and seven novel genes (referred to as LIP1, LIP2, LIP4, LIP5, LIP6, LIP7, LIP8) (see Table 1b). In addition to LYST 6578-7449, LIP6 interacted with the LYST domain containing WD40 repeats (bp 10576-11611). Two independent isolates of LIP6 were identified among interactions with the WD40 repeats. Casein kinase II beta subunit which interacted with LYST fragment 3190-4032 in forward screens, also interacted with the LYST WD40 repeat domain in reverse screens (see Table Ib).

The BEACH domain of LYST (bp 9502-10590) interacted with six proteins: the amino terminal third of atrophin-1 and embryonic Fyn-substrate 1 (Efs1), the amino terminus of OPAcontaining protein, heterogeneous nuclear riboprotein M4, LIP3 and LIP9 (see Table Ib). LIP3 also interacted with LYST 9037-9585. LIP3 interacted as well with LYST domain 9037-9585 which includes part of the BEACH domain.

The LYST fragment 9037-9585 which includes the 5' part of the BEACH domain was found to interact with LIP10, as detected in seven identical colonies in a forward screen of a heart library (Table Ia); with Glialblastoma cell differentiation-related protein (four identical isolates of a keratinocyte library); and with KB07 (five isolates representing two different clones of a heart library) (Table Ia).

As described in Table I and Example 3, infra, the inventors propose the following homologies for the ten novel LYST-IP proteins identified in the present invention, said homologies being based on BLAST 2.0 software program (N.C.B.I.) homology searches in known nucleic acid and amino acid databases: LIP1 (Tcp-10 homolog), LIP2 (L17 homolog), LIP3 (Roaz protein homolog), LIP4 (hnRNP-e2 homolog), LIP6 (Ns2-3 homolog), LIP7 (TCP10A homolog), LIP8 (KAP4L homolog). LIP9 (etr-1 homolog), and LIP10 (chicken tyrosine kinase homolog). No known homologies where identified for LIP5.

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The present invention relates to the novel nucleotide sequences of LYST-2. LIP-1, LIP-2, LIP-3. LIP-4. LIP-5, LIP-6. LIP-7. LIP-8. LIP-9. and LIP-10 genes and their encoded amino acid sequences. Said genes have not been previously described. and the protein translation products of said genes have not been previously defined. The invention provides for a LYST-2. LIP-1, LIP-2. LIP-3. LIP-4, LIP-5, LIP-6. LIP-7. LIP-8. LIP-9. or LIP-10 protein and gene encoding the protein from many different species, particularly vertebrates, and more particularly mammals. (The LYST-IP subgroup comprising the LIP-1, LIP-2, LIP-3, LIP-4, LIP-5. LIP-6. LIP-7, LIP-8, LIP-9. and LIP-10 proteins or genes encoding said proteins are designated herein as the ten "novel LIP" genes or proteins.) In a preferred embodiment, the ten novel LIP proteins and genes are of human origin. Production of the foregoing proteins and derivatives, e.g., by recombinant methods, is also provided in the present invention. The present invention further relates to a LYST-IP protein derivative or analog that is functionally active, i.e., capable of displaying one or more known functional activities associated with a full length (wild-type) LYST-IP protein. Functional activities include, but are not limited to, immunogenicity, antigenicity, and the ability to form a complex with LYST or LYST-2.

Derivatives, fragments, and analogs provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively. Fragments are, at most, one nucleic acid-less or one amino acid-less than the wild type full length sequence. Derivatives and analogs may be full length if said full length derivative or analog contains a modified nucleic acid or amino acid, as described infra. Derivatives or analogs of LYST or LYST-2 and LYST-IPs, include, but are not limited to, molecules comprising regions that are substantially homologous to LYST or LYST-2 or LYST-IPs, in various embodiments, by at least about 30% through about 95% identity (with a preferred identity of 90-95%) over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement (e.g., the inverse complement) of a sequence encoding LYST or LYST-2 or a LYST-IP under stringent (the preferred embodiment), moderately stringent, or low stringent conditions. See e.g. Ausubel, et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and infra.

Similarly, fragments of LYST or LYST-2 and LYST-IPs, include, but are not limited to. molecules comprising regions that are substantially homologous to LYST or LYST-2 or LYST-

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IPs, in various embodiments, by at least about 30% through about 95% identity over all entires acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement (e.g., the inverse complement) of a sequence encoding LYST or LYST-2 or a LYST-IP under stringent (the preferred embodiment), moderately stringent, or low stringent conditions. In a preferred embodiment of the present invention, the aforementioned fragments possess nucleotide or amino acid sequences which are comprised of at least 80% of the wild type, full length LYST or LYST-2 or LYST-IP proteins or 80% of the nucleic acid sequences encoding these proteins, with an overall 90% nucleic acid or amino acid sequence identity.

LYST:LYST-IP complexes, by virtue of the interaction, are implicated in modulating the functional activities of LYST or LYST-2 and its binding partners. Such functional activities include, but are not limited to, (i) physiological processes including, but not restricted to, signal transduction, vesicular transport, the formation and exocytosis of acidic intracellular organelles, and protein trafficking; and (ii) pathological processes including, but not restricted to, atopic diseases, autoimmune diseases, degenerative disorders including neurodegenerative disease, hyperproliferative disorders including tumorigenesis and tumor progression, oculocutaneous albinism, hypopigmentation and platelet dysfunction.

Structural aspects of LYST:LYST-IP complexes. LYST-2 protein, or said ten novel LIP proteins identified *supra*, and fragments, derivative, and analogs thereof, contain determinants important for antigenicity (defined herein as the ability to bind with an antibody specific to a designated protein or protein complex, or the ability to compete for binding with same), and immunogenicity (defined herein as the ability to generate an antibody that binds to a designated protein or protein complex). Derivatives or analogs of the LYST:LYST-IP complex that have the desired immunogenicity or antigenicity can be used in immunoassays, for immunization, for inhibition of LYST:LYST-IP complex activity, etc. Derivatives or analogs of the LYST:LYST-IP complex that retain or enhance, or alternatively lack or inhibit, a property of interest (*e.g.*, participation in a LYST:LYST-IP complex) can be used as inducers, or inhibitors, respectively. of such a property and its physiological correlates. A specific embodiment relates to a LYST:LYST-IP complex of a fragment of a LYST-2 protein and/or a fragment of a LYST-IP protein that can be bound by an anti-LYST and/or anti-LYST-IP antibody or by an antibody specific for a LYST:LYST-IP complex. when such fragment is included in a LYST:LYST-IP complex.

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The present invention is directed to methods of screening for proteins that interact with (e.g., bind to) LYST or LYST-2. The invention further relates to LYST or LYST-2 complexes, in particular LYST or LYST-2 complexes with one or more of the above said LYST-IP proteins. The invention further relates to complexes of derivatives, analogs and fragments of LYST or LYST-2, with derivatives, analogs and fragments thereof of said LYST-IPs. LYST:LYST-IP complexes may be comprised of a derivative of LYST or LYST-2 with a wild type LYST-IP, a wild type LYST or LYST-2 with a derivative LYST-IP, or a derivatives LYST or LYST-2 with a derivative LYST-IP, wherein said derivative has wild type function. In a preferred embodiment such complexes bind an anti-LYST:LYST-IP complex antibody. In a specific embodiment, complexes of human LYST or LYST-2 with human LYST-IP protein are provided.

The invention also provides methods of producing and/or isolating LYST:LYST-IP complexes. In a specific embodiment, the invention provides methods of using recombinant DNA techniques to express both LYST or LYST-2 and its binding partner (or fragments, derivatives or homologs of one or both members of the complex) either where both binding partners are under the control of one heterologous promoter (i.e., a promoter not naturally associated with the native gene encoding the particular complex component) or where each is under the control of a separate heterologous promoter.

The present invention provides for methods of diagnosis, prognosis, and screening for diseases and disorders associated with aberrant levels of one or more components of a group of proteins and protein complexes comprising: LYST:LYST-IP complexes and LYST-IP proteins. The invention also provides methods of treating or preventing such diseases or disorders, comprising administration of the LYST:LYST-IP complex or of the LYST-IP proteins, or of modulators of LYST:LYST-IP complex formation or activity (e.g., antibodies that bind the LYST:LYST-IP complex, or non-complexed LYST or its binding partner or a fragment thereof-preferably the fragment containing the portion of LYST or the LYST-IP that is directly involved in complex formation). These methods also include administering mutants of LYST or the LYST-IP that increase or decrease binding affinity, administering small molecule inhibitors/enhancers of LYST:LYST-IP complex formation, or administering antibodies that either stabilize or neutralize the LYST:LYST-IP complex.

Methods of assaying LYST:LYST-IP complexes or of assaying LYST-IP proteins for activity as therapeutics or diagnostics as well as methods of screening for LYST:LYST-IP complex or LYST-IP protein modulators (i.e., inhibitors, agonists and antagonists) are also provided. See, e.g., METHODS IN ENZYMOLOGY, (Abelson, Ed.). Academic Press, 1993.

### RECOMBINANT METHODOLOGIES

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For recombinant expression of one or more proteins, all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector. The necessary transcriptional and translational signals may be provided with the expression vector or may be supplied by the native promoter, and/or their flanking regions.

The LYST. LYST-2, or LYST-IP proteins either alone or in a complex, can be obtained by methods well known in the art for protein purification and recombinant protein expression. For recombinant expression of one or more of the proteins, the nucleic acid containing all or a portion of the nucleotide sequence encoding the protein can be inserted into an appropriate expression vector, *i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted protein coding sequence. The necessary transcriptional and translational signals can also be supplied by the native promoter for LYST or LYST-2 or any LYST-IP genes, and/or their flanking regions.

In a preferred embodiment, a LYST:LYST-IP complex is obtained by expressing the entire LYST or LYST-2 sequence and a LYST-IP coding sequence in the same cell, either under the control of the same promoter or under two separate promoters. In yet another embodiment, a derivative, fragment or homolog of LYST or LYST-2 and/or a derivative, fragment or homolog of a LYST-IP are recombinantly expressed. Preferably the derivative, fragment or homolog of LYST or of LYST-2 and/or of the LYST-IP protein forms a complex with a binding partner identified by a binding assay, such as the modified yeast two hybrid system (see *e.g.* Chien. *et al.*, 1991, *Proc Natl Acad Sci USA*. 88: 9578-9581), and more preferably forms a complex that binds to an anti-LYST:LYST-IP complex antibody.

In a specific embodiment, a vector is used that comprises a promoter operably linked to nucleotide sequences encoding LYST or LYST-2 and/or a LYST-IP, or a fragment, derivative or homolog thereof, one or more origins of replication, and optionally, one or more selectable markers (e.g., an antibiotic resistance gene). In a preferred embodiment, a vector is used that comprises a promoter operably linked to nucleotide sequences encoding both LYST or LYST-2 and a LYST-IP, one or more origins of replication, and optionally, one or more selectable markers.

Expression vectors or derivatives which can be used include, but are not limited to. human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors such as lambda phage; plasmids such as pBR322, pUC

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plasmid derivatives, or the pBlueScript vector (Stratagene, La Jolla, CA); and cosmid vectors. Methods used may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Any suitable transcription and translation elements may be used, and expression may be regulated by any promoter and or enhancer known in the art. Promoters sources which may be used include, but are not limited to, viral, mammalian, prokaryotic, and plant promoters, and promoter element from yeast and other fungi. Transcriptional control regions may exhibits tissue specificity or ubiquitous expression. Positive clones may be identified by any method known in art. See *e.g.* Augubel, *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1993.

The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the pBlueScript vector (Stratagene, La Jolla, CA). Insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically "polished" to ensure compatibility. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and the LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene may be modified by homopolymeric tailing. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

Promoters which may be used include but are not limited to the SV40 early promoter (Bernoist and Chambon, 1981. *Nature 290*: 304-310); the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980. *Cell 22*: 787-797); the Herpes thymidine kinase promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. USA 78*: 1441-1445); the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982. *Nature 296*: 39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. USA 75*: 3727-3731) or the tac promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. USA 80*: 21-25. see also "Useful Proteins from Recombinant Bacteria". In: *Scientific* 

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American 1980. 242: 79-94); plant expression vectors comprising the nopaline synthetase promoter (Herrar-Estrella et al., 1984, Nature 303: 209-213) or the cauliflower mosaic virus 35S RNA promoter (Garder et al., 1981, Nucleic Acids Res. 9: 2871), and the promoter of the photosynthetic enzyme ribulose bisphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310: 115-120); promoter elements from yeast and other fungi such as the Gal4 promoter, the alcohol dehydrogenase promoter, the phosphoglycerol kinase promoter, the alkaline phosphatase promoter; and the following animal transcriptional control regions that exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38: 639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50: 399-409; MacDonald 1987, Hepatology 7: 425-515), insulin gene control region which is active in pancreatic beta cells (Hanahan et al., 1985, Nature 315: 115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38: 647-658; Adams et al., 1985, Nature 318: 533-538; Alexander et al., 1987, Mol. Cell Biol. 7: 1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45: 485-495), albumin gene control region which is active in liver (Pinckert et al., 1987, Genes and Devel. 1: 268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5: 1639-1648; Hammer et al., 1987, Science 235: 53-58), alpha-1 antitrypsin gene control region which is active in liver (Kelsey et al., 1987, Genes and Devel. 1: 161-171), beta globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315: 338-340; Kollias et al., 1986, Cell 46: 89-94), myelin basic protein gene control region which is active in oligodendrocyte cells of the brain (Readhead et al., 1987, Cell 48: 703-712), myosin light chain-2 gene control region which is active in skeletal muscle (Sani 1985, Nature 314: 283-286), and gonadotrophic releasing hormone gene control region which is active in gonadotrophs of the hypothalamus (Mason et al., 1986, Science 234: 1372-1378).

In a specific embodiment, an expression vector containing the coding sequence, or a portion thereof, of LYST or LYST-2 and a LYST-IP either together or separately, is made by subcloning the gene sequences into the EcoRI restriction site of one of the three pGEX vectors (glutathione S-transferase expression vectors: Smith and Johnson, 1988, *Gene* 7: 31-40; Promega Corp., Madison, WI.). This allows for the expression of products in the correct reading frame.

Expression vectors containing the sequences of interest can be identified by three general approaches: (a) nucleic acid hybridization. (b) presence or absence of marker gene function, and (c) expression of the inserted sequences. In the first approach, LYST, LYST-2, 14-3-3 protein,

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HS1 protein, Hrs. BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta. Fte-1. ERRal, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I GBDR1, OPA containing protein. M4 protein. LIP1. LIP2. LIP3. LIP4. LIP5, LIP6, LIP7. LIP8. LIP9, or LIP10, or other LYST-IP sequences can be detected by nucleic acid hybridization to probes comprising sequences homologous and complementary to the inserted sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain marker functions (e.g., binding to an anti-LYST, anti-LYST-IP, or anti-LYST:LYST-IP complex antibody, resistance to antibiotics, occlusion body formation in baculovirus, etc.) caused by insertion of the sequences of interest in the vector. For example, if a LYST or LYST-2 or LYST-IP gene, or portion thereof, is inserted within the marker gene sequence of the vector, recombinants containing the LYST or LYST-2 or LYST-IP fragment will be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying for LYST, LYST-2, 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I. importin beta, Fte-1, hERRa1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, or other LYST-IP products expressed by the recombinant vector. Such assays can be based, for example, on the physical or functional properties of the interacting species in in vitro assay systems, e.g., formation of a LYST:LYST-IP complex or immunoreactivity to antibodies specific for the protein.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

Once a recombinant cell expressing LYST or LYST-2 and/or a LYST-IP protein, or fragment or derivative thereof, is identified, the individual gene product or complex can be isolated and analyzed. This is achieved by assays based on the physical and/or functional properties of the protein or complex, including, but not limited to, radioactive labeling of the product followed by analysis by gel electrophoresis, immunoassay, cross-linking to marker-labeled product, etc.

Once recombinant LYST, LYST-2, 14-3-3 protein. HS1 protein. Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERRa1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein. M4 protein. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, or other LYST-IP molecules is

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identified and the complexes or individual proteins are isolated, several methods known in the art can be used to propagate them. Once a suitable host system and growth conditions have been established, recombinant expression vectors can be propagated and amplified in quantity. As previously described, the expression vectors or derivatives which can be used include, but are not limited to, human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors such as lambda phage; and plasmid and cosmid vectors.

In addition, a host cell strain may be chosen that modulates the expression of the inserted sequence, or modifies or processes the expressed protein in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically-engineered LYST or LYST-2 and/or LYST-IP may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, phosphorylation, etc.) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein is achieved. For example, expression in a bacterial system can be used to produce an unglycosylated core protein, while expression in mammalian cells can ensure native glycosylation of a heterologous mammalian protein. Furthermore, different vector/host expression systems may effect processing reactions to different extents.

Additionally, the LYST or LYST-2 and/or LYST-IP encoding nucleotide sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, chemical mutagenesis and *in vitro* site-directed mutagenesis (Hutchinson *et al.*, 1978, *J. Biol. Chem 253*: 6551-6558), use of TAB<sup>TM</sup> linkers (Pharmacia), etc.

A variety of host-vector systems may be utilized to express the protein coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors or bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

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Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, prenylation, acetylation, phosphorylation) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the introduced protein is achieved. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used, see, e.g., supra.

Manipulations of LYST or LYST-2 and/or LYST-IP sequences may be made at the protein level (e.g. post-translational). Included within the scope of the invention are derivatives of complexes of LYST or LYST-IP fragments, derivatives or analogs thereof that are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, prenylation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to, specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.; acetylation; formylation; oxidation; reduction; or modification by metabolic processes.

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Due to the degeneracy of nucleotide coding sequences, other DNA sequences that encode substantially the same amino acid sequence as a LYST or LYST-2 or LYST-IP gene can be used in the practice of the present invention. Said nucleotide sequences may be altered by the substitution of different codons that encode the same amino acid residue within the sequence. thus producing a silent change. Substitute codons for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic), polar neutral, positively charged (basic), and negatively charged (acidic) amino acids.

Likewise, LYST or LYST-2 and LYST-IP derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of LYST or LYST-2 or a LYST-IP, including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar

(hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

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In specific embodiments, the LYST or LYST-2 and/or LYST-IP protein sequences are modified to include a fluorescent label or to have a heterofunctional reagent; such heterofunctional reagents may be used to crosslink the protein to other members of the complex or to other LYST-IPs, or if desired, contain non-classical amino acids or chemical amino acid analogs introduced as a substitution or addition into the LYST or LYST-2 and/or a LYST-IP. Non-classical amino acids include but are not limited to amino isobutyric acid, 3-amino propionoic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, fluoro-amino acids, C-methyl amino acids, N-methyl amino acids, designer amino acids and amino acid analogs in general. Furthermore, classical or non-classical amino acids can be D (dextrorotary) or L (levorotary).

In addition, analogs and derivatives of LYST or LYST-2 and/or a LYST-IP, or analogs and derivatives of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, can be chemically synthesized. For example, a peptide corresponding to a portion of LYST or LYST-2 and/or a LYST-IP, which comprises the desired domain or mediates the desired activity *in vitro* (*e.g.*, LYST:LYST-IP complex formation) can be synthesized by use of a peptide synthesizer. Furthermore, if desired, non-classical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the LYST or LYST-2 and/or a LYST-IP. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, amino isobutyric acid, 4-aminobutyric acid (4-Abu), 2-aminobutyric acid (2-Abu), 6-amino hexanoic acid (e-Ahx), 2-amino isobutyric acid (Aib), 3-amino propionoic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids. C-methyl amino acids, N-methyl amino acids, and amino acid analogs in general. Furthermore, classical or non-classical amino acids can be D (dextrorotary) or L (levorotary).

Any of the methods described *infra* for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate

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transcriptional/translational control signals and protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleotide sequences encoding LYST or LYST-2 and a LYST-IP, or a derivative, fragment or homolog thereof), may be regulated by a second nucleotide sequence so that the gene or gene fragment thereof is expressed in a host transformed with the recombinant DNA molecule(s). For example, expression of the proteins may be controlled by any promoter/enhancer known in the art. In a specific embodiment, the promoter is not native to the gene for LYST, LYST-2 or for LYST-IP.

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Chimeric genes comprising portions of LYST or LYST-2 and/or a LYST-IP, or LIP-1, LIP-2, LIP-3, LIP-4, LIP-5, LIP-6, LIP-7, LIP-8, LIP-9, or LIP-10, fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of LYST and/or a LYST-IP, or a fragment of LIP-1, LIP-2, LIP-3, LIP-4, LIP-5, LIP-6, LIP-7, LIP-8, LIP-9, or LIP-10 protein, of at least six amino acids. DNA fragments may be used to construct a chimeric gene in an expression vector using recombinant methodologies well known in the art.

In other specific embodiments, the LYST or LYST-2 and/or LYST-IP, or fragment, homolog or derivative thereof, may be expressed as a fusion or chimeric protein product comprising the protein, fragment, homolog, or derivative joined via a peptide bond to a heterologous protein sequence of a different protein. Such chimeric products can be made by ligating the appropriate nucleic acids encoding the desired amino acids to each other in the proper coding frame by methods known in the art, and expressing the chimeric products in a suitable host by methods commonly known in the art. Alternatively, such a chimeric product can be made by protein synthetic techniques, *e.g.*, by use of a peptide synthesizer.

In cases where natural products are suspected of being mutant or are isolated from new species, the amino acid sequence of LYST or LYST-2, or a LYST-IP isolated from the natural source, as well as those expressed *in vitro*, can be determined from analysis of the DNA sequence, or alternatively, by direct sequencing of the isolated protein. Such analysis may be performed by manual sequencing or through use of an automated amino acid sequencer.

The LYST, LYST-2, or LYST-IP proteins, either alone or in a complex, can be isolated and purified by standard methods known in the art, including but not restricted to column chromatography (e.g., ion exchange, affinity, gel exclusion, reversed-phase high pressure, fast protein liquid, etc.), differential centrifugation, differential solubility, or by any other standard technique used for the purification of proteins. Starting material may be either from natural

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sources or recombinant host cells expressing the complexes or proteins. Compositions comprising a LYST protein. a LYST-2 protein. a LYST-IP protein. or a LYST:LYST-IP complex may be purified to as little as 5% homogeneity, from 5% to 90% homogeneity, or from 70% to 100% homogeneity. Alternatively, a protein or its derivative can be synthesized by standard chemical methods known in the art (see. e.g., Hunkapiller et al., 1984, Nature 310: 105-111). Functional properties may be evaluated using any suitable assay known in the art. See. e.g., METHODS IN ENZYMOLOGY, (Abelson, Ed.), Academic Press, 1993.

Alternatively, once a LYST-IP or its derivative is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the chimeric gene from which it was encoded. As a result, the protein or its derivative can be synthesized by standard chemical methods known in the art (see, e.g., Hunkapiller et al., 1984, Nature 310: 105-111).

The LYST:LYST-IP complex, or LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, may also be analyzed by hydrophilicity analysis (Hopp and Woods, 1981, *Proc. Natl. Acad. Sci. USA 78*: 3824-3828). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of the proteins, and help predict their orientation to aid in the design of substrates for experimental manipulation, such as in binding experiments, antibody synthesis, etc. Secondary structural analysis can also be done to identify regions of the LYST or LYST-2 and/or a LYST-IP that assume specific structures (Chou and Fasman, 1974, *Biochemistry 13*: 222-223). Manipulation, translation, secondary structure prediction, hydrophilicity and hydrophobicity profiles, open reading frame prediction and plotting, and determination of sequence homologies, can be accomplished using computer software programs available in the art.

Other methods of structural analysis including but not limited to X-ray crystallography (Engstrom, 1974, *Biochem. Exp. Biol. 11*: 7-13), mass spectroscopy and gas chromatography (see, METHODS IN PROTEIN SCIENCE, J. Wiley and Sons, New York, 1997), and computer modeling (Fletterick and Zoller, eds., 1986, "Computer Graphics and Molecular Modeling", In: CURRENT COMMUNICATIONS IN MOLECULAR BIOLOGY, COLD SPRING HARBOR LABORATORY, Cold Spring Harbor Press. New York) can also be employed.

#### IDENTIFICATION AND ISOLATION OF NOVEL LYST-IPs

The present invention relates to LYST-2, LIP-1, LIP-2, LIP-3, LIP-4, LIP-5, LIP-6, LIP-7, LIP-8, LIP-9, LIP-10, LIP-11, LIP-12, or LIP-13 protein as well as derivatives, fragments. homologs and analogs thereof, wherein the native protein, fragment, derivative or analog of said

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novel proteins are from animals, (e.g., mouse, rat, pig, cow. dog, monkey, human, fly, or frog) or from plants. In other specific embodiments, the fragment, derivative or analog is functionally active, i.e., capable of exhibiting one or more functional activity associated with wild type LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, e.g., ability to bind LYST or LYST -2, immunogenicity, or antigenicity.

The nucleotide sequences encoding human LYST, 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, ERRa1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, and M4 protein, are known. GenBank accession numbers for said genes are listed in the BACKGROUND Section.

Homologs or paralogs of species other than human can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning, see, e.g., as described *infra*. Such hybridization conditions and techniques are well known in the art. Nucleic acids encoding LYST, LYST-2, and LYST-IPs disclosed herein can be obtained by any method known in the art, e.g., by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence and/or by cloning from a cDNA or genomic library using an oligonucleotide specific for the gene sequence, see e.g., as described *infra*.

The cloned LYST or LYST-2 or LYST-IP gene sequence can be modified by any of numerous strategies known in the art (Sambrook et al., 1989, MOLECULAR CLONING, A LABORATORY MANUAL, 2d Ed., Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York). The sequences can be cleaved at appropriate sites with restriction endonuclease(s), modified further by enzymatic manipulation if desired, isolated, and ligated in vitro.

LYST or LYST-2 and/or LYST-IP, or LYST-2, LIP-1, LIP-2, LIP-3, LIP-4, LIP-5, LIP-6, LIP-7, LIP-8, LIP-9, or LIP-10 derivatives can be made by altering their respective sequence by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences that encode substantially the same amino acid sequence as a LYST or LYST-2 or LYST-IP gene can be used in the practice of the present invention. Said nucleotide sequences may be altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Substitute codons for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the

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nonpolar (hydrophobic). polar neutral, positively charged (basic), and negatively charged (acidic) amino acids.

The LYST. LYST-2. or LYST-IP derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned LYST or LYST-2 or LYST-IP gene sequence can be modified by any of numerous strategies known in the art (Sambrook *et al.*, 1989, MOLECULAR CLONING, A LABORATORY MANUAL, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The sequences can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of a gene encoding a derivative or analog of LYST or LYST-2 or a LYST-IP, care should be taken to ensure that the modified gene retains the original translational reading frame, uninterrupted by translational stop signals.

Chimeric genes comprising portions of LYST or LYST-2 and/or a LYST-IP. or LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of LYST and/or a LYST-IP, or a fragment of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, of at least six amino acids.

In a specific embodiment, fusion proteins are provided that contain the interacting domains of the LYST or LYST-2 protein and a LYST-IP and/or, optionally, a hetero-functional reagent, such as a peptide linker between the two domains, where such a reagent promotes the interaction of the LYST or LYST-2 and LYST-IP binding domains. These fusion proteins may be particularly useful where the stability of the interaction is desirable (due to the formation of the complex as an intra-molecular reaction), for example in production of antibodies specific to the LYST:LYST-IP complex.

In particular, LYST or LYST-2 and/or LYST-IP, or LIP1, LIP2, LIP3, LIP4, LIP5. LIP6, LIP7, LIP8, LIP9. or LIP10 derivatives can be made by altering their respective sequence by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences that encode substantially the same amino acid sequence as a LYST or LYST-2 or LYST-IP gene can be used in the practice of the present invention. These include but are not limited, to a nucleotide sequence comprising all or a portion of LYST, LYST-2, or a LYST-IP gene that is altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change.

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In a specific embodiment of the invention, proteins consisting of or comprising a fragment of LYST or LYST-2 or a LYST-IP comprising at least 6 (continuous) amino acids of LYST or LYST-2 or a LYST-IP are provided. In other embodiments, the fragment consists of at least about 10, 20, 30, 40, or 50 amino acids of LYST or LYST-2 or a LYST-IP. In specific embodiments, such fragments are not larger than about 35, 100 or 200 amino acids. Derivatives or analogs of LYST or LYST-2 and LYST-IPs, include, but are not limited to, molecules comprising regions that are substantially homologous to LYST or LYST-2 or LYST-IPs, in various embodiments, by at least about 30%, and ranging up to 99% identity (with a preferred identity of 90-95%) over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art. In another embodiment, derivatives or analogs of LYST or LYST-2 and LYST-IPs may differ by as little as one chemical moiety or side group. Derivatives or analogs of LYST or LYST-2 and LYST-IPs whose encoding nucleic acid is capable of hybridizing to the complement (e.g., the inverse complement) of a sequence encoding LYST or LYST-2 or a LYST-IP under stringent, moderately stringent, or nonstringent conditions, as described supra, are also provided.

In a specific embodiment of the present invention, such LYST:LYST-IP complex, or LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, whether produced by recombinant DNA techniques, chemical synthesis methods, or by purification from native sources, include but are not limited to those containing as a primary amino acid sequence all or part of the amino acid sequences substantially, as well as fragments and other analogs and derivatives thereof, including proteins homologous thereto.

In cases where natural products are suspected of being mutant or are isolated from new species, the amino acid sequence of LYST or LYST-2, or a LYST-IP isolated from the natural source, as well as those expressed *in vitro*, or from synthesized expression vectors *in vivo* or *in vitro*, can be determined from analysis of the DNA sequence, or alternatively, by direct sequencing of the isolated protein. Such analysis may be performed by manual sequencing or through use of an automated amino acid sequenator.

## IDENTIFICATION AND ISOLATION OF NOVEL LIP GENES

The present invention relates to the nucleotide sequences encoding a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein. In specific embodiments, the LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 nucleic acid sequence comprises the sequence of SEQ ID NOS: 3, 5, 7, 9, 11, 13, 15, 17, 19, and 21, respectively, or a portion thereof,

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or a nucleotide sequence encoding, in whole or in part, a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6. LIP7, LIP8, LIP9, or LIP10 protein (e.g., a protein comprising the amino acid sequence of SEQ ID NOS: 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22, respectively, or a portion thereof). The invention provides purified nucleic acids consisting of at least 6 nucleotides (i.e., a hybridizable portion) of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 sequence. In other embodiments, the nucleic acids consist of at least about 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene sequence, or a full-length LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene sequence. In another embodiment, the nucleic acids are smaller than about 35, 200 or 500 nucleotides in length. Nucleic acids can be single or double stranded.

The invention also relates to nucleic acids hybridizable to or complementary to the foregoing sequences, in particular the invention provides the inverse complement to nucleic acids hybridizable to the foregoing sequences (*i.e.*, the inverse complement of a nucleic acid strand has the complementary sequence running in reverse orientation to the strand so that the inverse complement would hybridize with little or no mismatches to the nucleic acid strand; thus, for example, where the coding strand is hybridizable to a nucleic acid with no mismatches between the coding strand and the hybridizable strand, then the inverse complement of the hybridizable strand is identical to the coding strand). In specific aspects, nucleic acid molecules are provided which comprise a sequence complementary to (specifically are the inverse complement of) at least about 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene.

In a specific embodiment, a nucleic acid which is hybridizable to a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 nucleic acid sequence (e.g., having sequence SEQ ID NOS: 3, 5, 7. 9, 11, 13, 15, 17, 19, and 21, respectively), or to a nucleic acid sequence encoding a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein derivative (or a complement of the foregoing), under conditions of low stringency, is provided. By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA* 78: 6789-6792): Filters containing DNA are pretreated for 6 hours at 40°C in a solution containing 35% formamide. 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA. 0.1% PVP. 0.1% Ficoll, 1% BSA. and 500 μg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll. 0.2% BSA. 100 μg/ml salmon sperm DNA.

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10% (wt/vol) dextran sulfate. and 5-20 x 106 cpm 32P-labeled probe. Filters are incubated in hybridization mixture for 18-20 hours at 40°C, and then washed for 1.5 hours at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an additional 1.5 hours at 60°C. Filters are biotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film. Other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations). See, e.g., Ausubel et al. (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

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In another specific embodiment, a nucleic acid sequence which is hybridizable to a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 nucleic acid sequence (or a complement of the foregoing), or to a nucleic acid sequence encoding a derivative of the same, under conditions of high stringency is provided. It should be noted that the most preferred embodiment of the present invention utilizes high stringency hybridization conditions. By way of example and not limitation, procedures using such conditions of high stringency are as follows: Prehybridization of filters containing DNA is carried out for 8 hours to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 hours at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 x 106 cpm of 32P-labeled probe. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 minutes before autoradiography. Other conditions of high stringency which may be used are well known in the art. See, e.g., Ausubel et al. (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In another specific embodiment, a nucleic acid sequence which is hybridizable to a LIP1. LIP2, LIP3, LIP4, LIP5, LIP6. LIP7, LIP8. LIP9, or LIP10 nucleic acid sequence or to a nucleic acid sequence encoding a LIP1. LIP2. LIP3. LIP4, LIP5, LIP6. LIP7. LIP8. LIP9, or LIP10 derivative (or a complement of the foregoing) under conditions of moderate stringency is provided. For example, but not limited to, procedures using such conditions of moderate stringency are as follows: Filters containing DNA are pretreated for 6 hours at 55°C in a solution containing 6X SSC, 5X Denhardt's solution. 0.5% SDS and 100 µg/ml denatured salmon sperm

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DNA. Hybridizations are carried out in the same solution with 5-20 x 106 cpm <sup>32</sup>P-labeled probe. Filters are incubated in hybridization mixture for 18-20 hours at 55°C, and then washed twice for 30 minutes at 60°C in a solution containing 1X SSC and 0.1% SDS. Filters are blotted dry and exposed for autoradiography. Other conditions of moderate stringency which may be used are well-known in the art. Washing of filters is done at 37°C for 1 hour in a solution containing 2X SSC, 0.1% SDS. Other conditions of moderate stringency which may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

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Nucleic acid molecules encoding derivatives and analogs of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins (*supra*), or antisense nucleic acids to the same (see, *e.g.*, *infra*) are additionally provided. As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, and not the other contiguous portions of the LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 as a continuous sequence.

Within nucleotide sequences, potential open reading frames can be identified using the NCBI BLAST program ORF Finder available to the public. Because all known protein translation products are at least 60 amino acids or longer (Creighton, 1992, PROTEINS, 2nd Ed., W.H. Freeman and Co., New York), only those ORFs potentially encoding a protein of 60 amino acids or more are considered. If an initiation methionine codon (ATG) and a translational stop codon (TGA, TAA, or TAG) are identified, then the boundaries of the protein are defined. Other potential proteins include any open reading frames that extend to the 5' end of the nucleotide sequence, in which case the open reading frame predicts the C-terminal or core portion of a longer protein. Similarly, any open reading frame that extends to the 3' end of the nucleotide sequence predicts the N-terminal portion of a longer protein. This methodology was used to identify open reading frames encoding the interactant LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10

Any method available in the art can be used to obtain a full length (i.e., encompassing the entire coding region) cDNA clone encoding a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9, or LIP10 protein. In particular, the polymerase chain reaction (PCR) can be used to amplify sequences in silico from a cDNA library. Oligonucleotide primers that hybridize to

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sequences at the 3° and 5° termini of the identified sequences can be used as primers to amplify by PCR sequences from a nucleic acid sample (cDNA or DNA), preferably a cDNA library, from an appropriate source (e.g., the sample from which the initial cDNA library for the modified yeast two hybrid assay fusion population was derived, or, e.g. the genomic DNA of the same).

PCR can be carried out. e.g., by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase. The DNA being amplified can include genomic DNA or cDNA sequences from any eukaryotic species. One can choose to synthesize several different degenerate primers for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions to amplify nucleic acid homologs (e.g., to obtain LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 sequences from species other than humans, or to obtain human sequences with homology to LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9, or LIP10) by allowing for greater or lesser degrees of nucleotide sequence similarity between the known nucleotide sequence and the nucleic acid homolog being isolated.

After successful amplification of a nucleic acid containing all or a portion of a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 sequence, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*. In this fashion, the nucleotide sequence of the entire LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene, as well as additional genes encoding a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein or analog, may be identified.

Any eukaryotic cell potentially can serve as the nucleic acid source for the molecular cloning of a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene. The nucleic acids can be isolated from vertebrates, including human and other primate sources, porcine, bovine, feline, avian, equine, canine, as well as additional mammalian sources, and insects. plants, etc. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (see, for example, Sambrook et al., 1989, MOLECULAR CLONING, A LABORATORY MANUAL, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; and Glover (ed.), 1985, DNA CLONING: A PRACTICAL APPROACH, MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA may contain regulatory and intronic DNA regions in addition to coding regions: clones

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derived from cDNA will contain only exon sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and/or polyacrylamide gel electrophoresis, and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, a portion of the LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene (of any species) (e.g., a PCR amplification product obtained as described above, or an oligonucleotide having a sequence of a portion of the known nucleotide sequence) or its specific RNA, or a fragment thereof, may be purified and labeled, and the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton and Davis, 1977, Science 196: 180-182; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72: 3961-3964). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is available, or by DNA sequence analysis and comparison to the known nucleotide sequence of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, e.g., has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, or antigenic properties or ability to bind LYST, as is known for LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10. If an anti-LIP1, anti-LIP2, anti-LIP3, anti-LIP4, anti-LIP5, anti-LIP6, anti-LIP7, anti-LIP8, anti-LIP9 or anti-LIP10 antibody is available, the protein may be identified by binding of labeled antibody to the putative LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 synthesizing clones, in an

ELISA (enzyme-linked immunosorbent assay)-type procedure.

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An alternative to isolating a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 cDNA includes, but is not limited to, chemically synthesizing the gene sequence itself from a known sequence. Other methods are possible and within the scope of the invention. The identified and isolated nucleic acids can then be inserted into an appropriate cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 nuclear acid sequence provided by the present invention includes those nucleotide sequences encoding substantially the same amino acid sequence as found in the respective native protein, those encoded amino acid sequences with functionally equivalent amino acids, and those sequences encoding other LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 derivatives or analogs, as described *supra*.

# ANTIBODIES TO LYST:LYST-IP COMPLEXES, AND LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, AND LIP10 PROTEINS

According to the present invention, the LYST:LYST-IP complex, or fragments. derivatives or homologs thereof, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein or fragments, homologs and derivatives thereof, may be used as immunogens to generate antibodies which immunospecifically bind such immunogens. Said antibodies include but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and Fab expression libraries. In a specific embodiment, antibodies to complexes of human LYST or LYST-2 and a human LYST-IP are produced. In another embodiment, complexes formed from fragments of a LYST or LYST-2 and a LYST-IP, where the fragments contain the protein domain that interacts with the other member of the complex, are used as immunogens for antibody production. In another specific embodiment, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins or fragments, derivatives, or homologs thereof are used as immunogens.

Various procedures known in the art may be used for the production of polyclonal antibodies to a LYST-IP complex, or to a derivative or analog thereof, or to a LYST-2.

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LIP1. LIP2. LIP3. LIP4. LIP5. LIP6. LIP7. LIP8. LIP9, or LIP10 protein, or derivative, fragment or analog thereof.

For production of the antibody, various host animals can be immunized by injection with the native LYST:LYST-IP complex, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, or a synthetic version, or a derivative of the foregoing, such as a cross-linked LYST:LYST-IP. Such host animals include but are not limited to rabbits, mice, rats, etc. Various adjuvants can be used to increase the immunological response, depending on the host species, and include but are not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, and potentially useful human adjuvants such as bacille Calmette-Guerin (BCG) and Corynebacterium parvum.

For preparation of monoclonal antibodies directed towards a LYST:LYST-IP complex or to a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, or derivatives, fragments or analogs thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. Such techniques include but are not restricted to the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256: 495-497), the trioma technique (Rosen et al., 1977, Cell 11: 139-147), the human Bcell hybridoma technique (Kozbor et al., 1983, Immunology Today 4: 72), and the EBV hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, In: Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals (See International Application No. PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80: 2026-2030), or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). In fact, according to the invention, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, Proc. Natl. Acad. Sci. USA 81: 6851-6855; Neuberger et al., 1984, Nature 312: 604-608; Takeda et al., 1985, Nature 314: 452-454) by splicing the genes from a mouse antibody molecule specific for the LYST:LYST-IP complex or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, together with genes from a human antibody molecule of appropriate biological activity, can be used; such antibodies are within the scope of this invention.

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According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce LYST:LYST-IP complex-specific and a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein-specific single chain antibody. An additional embodiment of the invention utilizes techniques described for the construction of Fab expression libraries (Huse *et al.*, 1989, *Science 246*: 1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for the LYST:LYST-IP complex, or an individual LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, derivative or analog. Non-human antibodies can be humanized by known methods (see, *e.g.*, U.S. Patent No. 5,225,539).

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Antibody fragments that contain the idiotypes of a LYST:LYST-IP complex or of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein can be generated by techniques known in the art. For example, such fragments include but are not limited to: the F(ab)<sub>2</sub> fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments that can be generated by reducing the disulfide bridges of the F(ab)<sub>2</sub> fragment; the Fab fragments that can be generated by treating the antibody molecular with papain and a reducing agent; and Fv fragments.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g., ELISA (enzyme-linked immunosorbent assay). To select antibodies specific to a particular domain of the LYST:LYST-IP complex, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, one may assay generated hybridomas for a product that binds to the fragment of the LYST:LYST-IP complex, or theLYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, that contains such a domain. For selection of an antibody that specifically binds a LYST:LYST-IP complex but which does not specifically bind to the individual proteins of the LYST-IP complex, one can select on the basis of positive binding to the LYST-IP complex and a lack of binding to the individual LYST and LYST-IP proteins.

Antibodies specific to a domain of the LYST:LYST-IP complex are also provided, as are antibodies to specific domains of the LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein.

The foregoing antibodies can be used in methods known in the art relating to the localization and/or quantitation of a LYST:LYST-IP complex or of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein of the invention, e.g., for imaging these

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proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

In another embodiment of the invention, anti-LYST:LYST-IP complex antibodies and fragments thereof, or anti-LYST-2, anti-LIP1, anti-LIP2, anti-LIP3, anti-LIP4, anti-LIP5, anti-LIP6, anti-LIP7, anti-LIP8, or anti-LIP9, or anti-LIP10 antibodies or fragments thereof, containing the binding domain, are therapeutics, as described *infra*.

#### DIAGNOSTICS, PROGNOSTICS, AND SCREENING

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LYST:LYST-IP complexes may be markers of normal physiological processes including, but not limited to, the physiological processes including signal transduction, vesicular transport, the formation and exocytosis of acidic intracellular organelles, and protein trafficking; and pathological processes including but not restricted to, atopic diseases, autoimmune diseases, degenerative disorders including neurodegenerative disease, hyperproliferative disorders including tumorigenesis and tumor progression, oculocutaneous albinism and hypopigmentation and platelet dysfunction, and thus have diagnostic utility. Further, definition of particular groups of patients with elevations or deficiencies of a LYST:LYST-IP complex, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, can lead to new classifications of diseases, furthering diagnostic ability.

Detecting levels of LYST:LYST-IP complexes, or individual proteins that have been shown to form complexes with LYST or LYST-2, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins; or detecting levels of mRNAs encoding components of the LYST:LYST-IP complexes, or mRNAs encoding a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, or LIP9 protein, may be used in prognosis, to follow the course of disease state, to follow therapeutic response, etc.

LYST:LYST-IP complexes and the individual components of the LYST:LYST-IP complexes, and derivatives, analogs and subsequences thereof; LYST or LYST-2 and/or LYST-IP, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 nucleic acids (and sequences complementary thereto); anti-LYST:LYST-IP complex antibodies and antibodies directed against the individual components that can form LYST:LYST-IP complexes; and antibodies specific to LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, have uses in diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders, and treatment

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thereof, characterized by aberrant levels of LYST:LYST-IP complexes, or by aberrant levels of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein.

In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti-LYST:LYST-IP complex antibody, or an antibody specific to LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding that occurs by such an antibody. In a specific aspect, such binding of antibody, for example in tissue sections, can be used to detect aberrant LYST:LYST-IP complex formation, or aberrant LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein localization, or aberrant (e.g., high, low or absent) levels of LYST:LYST-IP complex or complexes, or aberrant levels of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein. In a specific embodiment, an antibody against a LYST:LYST-IP complex can be used to assay a patient tissue or serum sample for the presence of the LYST:LYST-IP complex, where an aberrant level of the LYST:LYST-IP complex is an indication of a disease condition. In another embodiment, an antibody against LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 can be used to assay a patient tissue or serum sample for the presence of said protein, where an aberrant level of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 is an indication of a disease condition. By "aberrant levels" is meant increased or decreased levels relative to levels previously found present, or to a standard level in an analogous sample either from another portion of a body or from a subject not having the disorder. Immunoassays that can be used are described infra.

Nucleic acids encoding the components of the LYST:LYST-IP complexes and nucleic acids encoding a LYST-2, LIP1, LIP2. LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9, or LIP10 protein, and related nucleotide sequences and subsequences, including complementary sequences. can also be used in hybridization assays. The LYST or LYST-2 and/or LYST-IP nucleotide sequence, or a subsequence thereof, comprising about at least 6 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant levels of the mRNAs encoding the components of a LYST:LYST-IP complex, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, as described *supra*. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to LYST or LYST-2 or a LYST-IP DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any

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resulting hybridization. See *supra* for hybridization conditions. In a preferred aspect, the hybridization assay is carried out using nucleic acid probes capable of hybridizing to LYST or LYST-2 and to a binding partner of LYST or LYST-2 to measure concurrently the expression of both members of a LYST:LYST-IP complex. In another preferred embodiment, the expression of mRNAs encoding LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 is measured.

In specific embodiments, diseases and disorders involving or characterized by aberrant levels of LYST:LYST-IP complexes can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting aberrant levels of a LYST:LYST-IP complex, or un-complexed LYST or LYST-2 and/or a LYST-IP protein or nucleic acids or functional activity, including but not restricted to, binding to an interacting partner, or detecting mutations in LYST or LYST-2 and/or in a LYST-IP RNA, DNA or protein (e.g., translocations, truncations, changes in nucleotide or amino acid sequence relative to wild-type LYST and/or LYST-IP) that cause increased or decreased expression or activity of a LYST:LYST-IP complex and/or LYST or LYST-2 and/or protein that binds to LYST or LYST-2. Such diseases and disorders include but are not limited to those described in later subsections, infra.

By way of example, levels of a LYST:LYST-IP complex or the individual components of a LYST:LYST-IP complex can be detected by immunoassay; levels of LYST and/or of LYST-IP mRNA can be detected by hybridization assays (e.g., Northern blots, dot blots); binding of LYST or to a LYST-IP can be measured by binding assays commonly known in the art, translocations and point mutations in LYST or LYST-2 and/or in genes encoding a LYST-IP can be detected by Southern blotting, RFLP analysis, PCR using primers that preferably generate a fragment spanning at least most of the LYST or LYST-2 and/or LYST-IP gene, sequencing of the LYST or LYST-2 and/or LYST-IP gene, the latest translocation of the LYST or LYST-2 and/or LYST-IP gene, sequencing of the LYST or LYST-2 and/or LYST-IP genomic DNA or cDNA obtained from the patient, etc.

Assays well known in the art (e.g., assays described supra such as nucleic acid hybridization assays, activity assays, and infra such as immunoassays, etc.) can be used to determine whether one or more particular LYST:LYST-IP complexes are present at either increased or decreased levels, or are absent, in samples from patients suffering from a particular disease or disorder, or having a predisposition to develop such a disease or disorder as compared to the levels in samples from subjects not having such a disease or disorder.

Additionally, these assays can be used to determine whether the ratio of the LYST:LYST-IP complex to the un-complexed components of the LYST:LYST-IP complex, i.e., LYST or

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LYST-2 and/or the specific LYST-IP in the complex of interest, is increased or decreased in samples from patients suffering from a particular disease or disorder, or having a predisposition to develop such a disease or disorder, as compared to the ratio in samples from subjects not having such a disease or disorder.

In the event that levels of one or more particular LYST:LYST-IP complexes are determined to be altered in patients suffering from a particular disease or disorder, or having a predisposition to develop such a disease or disorder, then the particular disease or disorder or predisposition for a disease or disorder can be diagnosed, have prognosis defined for, be screened for, or be monitored by detecting altered levels of the one or more LYST:LYST-IP protein complexes, the mRNA that encodes the members of the one or more particular LYST:LYST-IP complexes, or LYST:LYST-IP complex functional activity.

Accordingly, in a specific embodiment of the invention, diseases and disorders involving increased levels of one or more LYST:LYST-IP complexes can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting increased levels of the one or more LYST:LYST-IP complexes, the mRNA encoding both members of the complex, or complex functional activity, or by detecting mutations in LYST or LYST-2 or the LYST-IP (e.g., translocations in nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type LYST or LYST-2 or LYST-IP) that stabilize or increase LYST:LYST-IP complex formation.

Accordingly, in another specific embodiment of the invention, diseases and disorders involving decreased levels of one or more LYST:LYST-IP complexes can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting decreased levels of the one or more LYST:LYST-IP complexes, the mRNA encoding the members of the one or more complexes, or complex functional activity, or by detecting mutations in LYST or LYST-2 or the LYST-IP (e.g., translocations in nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type LYST or LYST-2 or the LYST-IP) that inhibit or reduce LYST:LYST-IP complex formation.

In yet another specific embodiment, diseases and disorders involving aberrant expression of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein are diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting aberrant levels of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins, or mRNA, or functional activity, or by detecting

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mutations in LYST-2. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein or mRNA or DNA (e.g., translocations in nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type LYST-2 or a wild-type novel LIP gene or protein) that cause aberrant expression or activity of said LYST-2 or said novel LIP protein. Such diseases and disorders include but are not limited to those described *infra*. By way of example, levels of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 mRNA or protein, LYST or LYST-2 binding activity, or the presence of translocations or point mutations, can be determined as de tibed above.

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Assays well known in the art (e.g., assays described above such as immunoassays, nucleic acid hybridization assays, activity assays, etc.) can be used to determine whether LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 are present at either increased or decreased levels, or are absent, in samples from patients suffering from a particular disease or disorder, or having a predisposition to develop such a disease or disorder, as compared to the levels in samples from subjects not having such a disease or disorder.

In the event that levels of said LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 are determined to be altered in patients suffering from a particular disease or disorder, or having a predisposition to develop such a disease or disorder, then the particular disease or disorder or predisposition for a disease or disorder can be diagnosed, have its prognosis determined, be screened for, or be monitored by detecting altered levels of protein, mRNA, or functional activity (e.g., binding to LYST) of said LYST-2 or novel LIP.

Accordingly, in a specific embodiment of the invention, diseases and disorders involving increased levels of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting increased levels of a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, encoding nucleic acids, or functional activity, or by detecting mutations in LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 (e.g., translocations in nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type LYST-2 or novel LIP) that enhance said LYST-2 or novel LIP stability or functional activity.

Accordingly, in another specific embodiment of the invention, diseases and disorders involving decreased levels of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting decreased levels of LIP1, LIP2, LIP3, LIP4,

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LIP5. LIP6. LIP7. LIP8. LIP9. or LIP10 protein. nucleic acids. or functional activity, or by detecting mutations in LYST-2. LIP1. LIP2. LIP3, LIP4. LIP5. LIP6. LIP7. LIP8. LIP9, or LIP10 (e.g., translocations in nucleic acids. truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type LYST-2 or novel LIP) that destabilize or reduce LYST-2, LIP1. LIP2. LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9, or LIP10 functional activity.

The use of detection techniques, especially those involving antibodies against LYST:LYST-IP complexes, or against a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, provides a method of detecting specific cells that express the complex or protein. Using such assays, specific cell types can be defined in which one or more particular LYST:LYST-IP complex, or said individual LYST-2 or novel LIP protein, is expressed, and the presence of the complex or protein can be correlated with cell viability.

Also embodied are methods to detect a LYST:LYST-IP complex, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, in cell culture models that express particular LYST:LYST-IP complexes or said individual LYST-2 or novel LIP proteins, or derivatives thereof, for the purpose of characterizing or preparing LYST:LYST-IP complexes, or said individual LYST-2 or novel LIP proteins for harvest. This embodiment includes cell sorting of prokaryotes such as, but not restricted to, bacteria (Davey and Kell, 1996, Microbiol. Rev. 60: 641-696), primary cultures and tissue specimens from eukaryotes, including mammalian species such as human (Steele et al., 1996, Clin. Obstet. Gynecol 39: 801-813), and continuous cell cultures (Orfao and Ruiz-Arguelles, 1996, Clin. Biochem. 29: 5-9). Such isolations can also be used as methods of diagnosis, described supra.

Kits for diagnostic use are also provided that comprise in one or more containers an anti-LYST:LYST-IP complex antibody or an antibody specific to LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, and, optionally, a labeled binding partner to the antibody. Alternatively, the anti-LYST:LYST-IP complex antibody, or antibody specific to LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10. can be labeled with a detectable marker, e.g., a chemiluminescent, enzymatic, fluorescent, or radioactive moiety. A kit is also provided that comprises in one or more containers a nucleic acid probe capable of hybridizing to LYST or LYST-2 and/or a LYST-IP mRNA. In a specific embodiment, a kit can comprise in one or more containers a pair of primers (e.g., each in the size range of about 6-40 nucleotides) that are capable of priming amplification [e.g., by polymerase chain reaction (see e.g., Innis et al., 1990, PCR PROTOCOLS, Academic Press, Inc., San Diego, CA), ligase chain

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reaction (see EP 320,308), use of QB replicase, cyclic probe reaction, or other methods known in the art], under appropriate reaction conditions of at least a portion of a LYST and/or a LYST-IP, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 nucleic acid sequence. A kit can optionally further comprise in a container a predetermined amount of a purified LYST:LYST-IP complex. LYST or LYST-2 and/or a LYST-IP, or said individual LYST-2 or novel LIP protein, or an encoding nucleic acid molecule thereof, e.g., for use as a standard or control.

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## THERAPEUTIC USES OF LYST:LYST-IP COMPLEXES AND LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, OR LIP9, OR LIP10

The invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic compound (termed herein "Therapeutic"). Such "Therapeutics" include but are not limited to: LYST:LYST-IP complexes, LYST and the individual LYST-IP proteins and analogs and derivatives (including fragments) of the foregoing (e.g., as described herein above); antibodies there to (as described herein above); nucleic acids encoding LYST or LYST-2 and/or a LYST-IP, and analogs or derivatives thereof (e.g., as described herein above); LYST or LYST-2 and/or LYST-IP antisense nucleic acids, and LYST:LYST-IP complex and LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 modulators (i.e., inhibitors, agonists and antagonists).

As reviewed *supra*, LYST and LYST-2 are centrally implicated in physiological processes including but not limited to signal transduction, vesicle morphology, the formation and exocytosis of acidic intracellular organelles, and sorting of proteins which is necessary for function of intracellular organelles. Likewise, LYST has been strongly implicated in pathological conditions, including but not limited to, atopic diseases (including bronchial asthma), severe immunologic deficiency with deficient functions of granulocytes, lymphocytes and natural killer cells, oculocutaneous albinism, hypopigmentation, platelet dysfunction (bleeding diathesis), neurodegeneration such as peripheral neuropathy and ataxia, and cancer. Likewise, LYST has been strongly implicated in protection from pathological conditions including but not limited to autoimmune disorders (including systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease).

Most of the known LYST or LYST-2 interactants described in the present invention are likewise involved in signal transduction and secretion processes including calcium-dependent exocytosis, see BACKGROUND Section. *supra*. This relates to particularly 14-3-3 protein, HS1

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protein. Hrs. BMK1. KB07. Efs. XAP-4, OS9. HRS. calmodulin. Troponin I, Fte-1. casein kinase II subunit beta, and norbin. A linkage between the signal transduction disorders and induction of apoptosis exists, which may relate to LYST, LYST-2, and particularly the interactants 14-3-3 protein, HS1 protein, Efs (Fyn-associated substrate), HRS. calmodulin. casein kinase II subunit beta, and norbin.

LYST or LYST-2 interactants found in this invention with a role in autoimmune-diseases and inflammation are particularly the estrogen-receptor related protein, 14-3-3 proteins, and Imogen 38. The estrogen-receptor related protein is expressed in human arthritic synovium, and estrogen modulates local inflammation in various diseases. 14-3-3 proteins play a role in autoimmune-diseases, for example, by interacting with insulin receptor substrate 1. Imogen 38 is an mitochondrial autoantigen which is a target for autoimmune attack in diabetes.

Furthermore. LYST and LYST-2 and most of its binding partners, as identified herein (e.g., 14-3-3 protein, HS1 protein, Hrs, calmodulin, Efs. Importin beta, Atrophin-1, HBF-G2, and DGS-1) are significantly implicated in disorders of neurodegeneration, see BACKGROUND Section, supra. 14-3-3 is present in Alzheimer's Disease neurofibrillary tangles and in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. The ATPase Hrs is implicated in calcium-regulated secretion. Calmodulin is involved in neurotransmitter release and activated exocytosis in neuroendocrine cells. The phosphoprotein Efs is associated with the tyrosine-kinase Fyn, which is involved in many signal transduction processes, including those in neuronal cells. Importin beta is a nuclear protein involved in nuclear protein translocation. Atrophin-I is the protein product of the gene implicated in dentatorubral pallidoluysian atrophy (DRPLA, Smith's disease) and is ubiquitously expressed in neuronal tissues. The LYST-2 interactant HBF-G3 is a brain factor expressed in fetal tissues and is likely to be involved in development and embryogenesis. DGS-I is the protein associated with DiGeorge syndrome, a developmental defect which is characterized by hypoplasia or aplasia of the thymus and parathyroids, facial dysmorphism and conotruncal cardiac malformations.

Disorders of cell cycle progression, cell differentiation, and transcriptional control. including cancer and tumorigenesis and tumor progression can involve LYST, LYST-2. and particularly the interactants 14-3-3 proteins, HS1 protein, and estrogen-receptor related protein. Chediak-Higashi patients have an increased tumor growth rate and higher metastatic frequency. In a large Japanese cohort, non-Hodgkin's lymphoma occurred in over one third of CHS patients (Hayakawa et al., 1986, Jpn. J. Cancer Res. 77: 74-79). The effect of the LYST protein on tumorigenesis may be due to the involvement of natural killer cells in cancer. Norbin may be

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accompanied with neurite-outgrowth in neuro-2a cells and may play a role in the formation of new synapses. Thus, it may be involved in disorders of neurodegeneration. Importin beta subunit is implicated in cellular response to viral infection, including HIV-1 (AIDS).

Most of the novel LYST interactants encoded by the genes for LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 can also be related to the implicated functions of LYST and LYST-2 and the interactants described above.

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Two interactants, LIP1 and LIP7, have homologies to human T-complex protein (Tcp)-10 and also to cytoskeletal and vesicular proteins. In mouse, Tcp-10 genes are expressed exclusively in male germ cells during spermatogenesis. Tcp-10 has a conserved C-terminal region with unique nonapeptide repeat and a conserved N-terminal region containing a pair of leucine zippers within a region that shows additional similarity to the coiled-coil regions of various cytosolic polypeptides (Islam et al., Human Mol. Genet. 2: 2075-2079).

At least two interactants, LIP2 and LIP9, may have nuclear function. LIP2 is homologous to ribosomal protein L17. LIP9 is homolog to the Xenopus laevis elav-type ribonucleoprotein Etr-1, a putative RNA-binding protein found in neural tissue, and thus, LIP9 might be involved in neurodegenerative diseases.

Another interactant, LIP3, may be involved in disorders of neurodegeneration. LIP3 is homolog to rat O/E-1-associated zinc finger protein (Roaz), that plays a role in regulating the temporal and spatial pattern of olfactory neuronal-specific gene expression. Roaz protein functions by interacting with the olfactory factor O/E-1 and modulating its transcriptional activity. The Roaz mRNA was found in brain, eye, olfactory epithelium, spleen, and heart and was expressed in the basal layer, consisting of neural precursor cells and immature sensory neurons of the olfactory epithelium, but not in the mature receptor cells (Tsai & Reed, 1997, J. Neurosci. 17: 4159-4169).

LIP-8 is the human homolog of the rat norbin gene. Rat norbin is expressed in the brain and is induced by treatment of tetraethylammonium in rat hippocampal slice accompanied with neurite-outgrowth in neuro-2a cells. The neurite-outgrowth-related norbin protein may play a role in neural plasticity because of the formation of new synapses.

## TREATMENT OF DISEASES AND DISORDERS WITH ALTERED LYST:LYST-IP OR LYST LEVELS

A wide range of cell diseases affected by intracellular signal transduction, vesicle transport and protein trafficking can be treated or prevented by administration of a Therapeutic

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that modulates (*i.e.*, inhibits, antagonizes, enhances or promotes) LYST:LYST-IP complex activity. All of these disorders can be treated or prevented by administration of a Therapeutic that modulates (*i.e.*, inhibits, antagonizes, enhances or promotes) LYST:LYST-IP complex activity, or modulates LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 activity.

Diseases or disorders associated with aberrant levels of LYST:LYST-IP complex levels or activity, or aberrant levels of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, may be treated by administration of a Therapeutic that modulates LYST:LYST-IP complex formation or activity, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 activity. In a specific embodiment, the activity or level of LYST is modulated by administration of a LYST-IP. In another specific embodiment, the activity or level of a LYST-IP is modulated by administration of LYST or LYST-2.

Diseases and disorders associated with underexpression of a LYST:LYST-IP complex, or LYST or LYST-2 or a particular LYST-IP, are treated or prevented by administration of a Therapeutic that promotes (*i.e.*, increases or supplies) LYST:LYST-IP complexes or function. Examples of such a Therapeutic include but are not limited to LYST:LYST-IP complexes and derivatives, analogs and fragments thereof that are functionally active (*e.g.*, active to form LYST:LYST-IP complexes), un-complexed LYST or LYST-2 and LYST-IP proteins, and derivatives, analogs, and fragments thereof, and nucleic acids encoding the members of a LYST:LYST-IP complex, or functionally active derivatives or fragments thereof (*e.g.*, for use in gene therapy). In a specific embodiment are derivatives, homologs or fragments of LYST or LYST-2 and/or a LYST-IP that increase and/or stabilize LYST:LYST-IP complex formation. Examples of other agonists can be identified using *in vitro* assays or animal models, examples of which are described herein.

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## **ALTERED COMPLEX FORMATION OR ACTIVITY**

Diseases and disorders characterized by increased (relative to a subject not suffering from the disease or disorder) LYST:LYST-IP levels or activity, or increased LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 levels or activity, can be treated with Therapeutics that antagonize (*i.e.*, reduce or inhibit) LYST:LYST-IP complex formation or activity, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 levels or activity. Therapeutics that can be used, include but are not limited to. LYST or LYST-2 or a LYST-IP, or analogs, derivatives or fragments thereof; anti-LYST:LYST-IP complex antibodies

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(e.g., antibodies specific for LYST:14-3-3 protein, LYST:HS1 protein, LYST:Hrs. LYST:BMK1, LYST:KB07, LYST:Efs, LYST:OS9, LYST:casein kinase II beta SU, LYST:calmodulin, LYST:troponin I, LYST:Importin beta, LYST:Fte-1, LYST:estrogen-receptor related protein, LYST:Imogen 38, LYST:Atrophin-1, LYST:norbin, LYST:GBDR1, LYST:OPA containing protein, LYST:M4 protein, LYST:LIP1, LYST:LIP2, LYST:LIP3, LYST:LIP4, LYST:LIP5, LYST:LIP6, LYST:LIP7, LYST:LIP8, LYST:LIP9, LYST:LIP10, LYST-2:HBF-G2, LYST-2:14-3-3, LYST-2:XAP-4 complexes), and antibodies specific to LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, fragments and derivatives thereof containing the binding region thereof; nucleic acids encoding LYST or LYST-2 or a LYST-IP; concurrent administration of LYST or LYST-2 and LYST-IP antisense nucleic acids or of LYST-2 or individual novel LIP antisense nucleic acids, or of LYST or LYST-2 and LYST-IP nucleic acids or of individual LYST-2 or novel LIP nucleic acids that are dysfunctional (e.g., due to a heterologous (non-LYST or LYST-2 and/or non-LYST-IP) insertion within the coding sequences of the LYST or LYST-IP coding sequences)) that are used to "knockout" endogenous LYST or LYST-2 and/or LYST-IP function by homologous recombination (see, e.g., Capecchi, 1989, Science 244: 1288-1292).

In a specific embodiment of the invention, a nucleic acid containing a portion of a LYST or LYST-2 and/or a LYST-IP gene in which the LYST or LYST-2 and/or LYST-IP sequences flank (are both 5' and 3' to) a different gene sequence, is used as a LYST or LYST-2 and/or a LYST-IP antagonist, or to promote LYST or LYST-2 and/or LYST-IP inactivation by homologous recombination (see also Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA 86*: 8932-8935. Zijlstra et al., 1989, *Nature 342*: 435-438). Additionally, mutants or derivatives of a first LYST-IP protein that have greater affinity for LYST than a second LYST-IP may be administered to compete with the second LYST-IP protein for LYST binding, thereby reducing the levels of LYST complexes with the second LYST-IP. Other Therapeutics that inhibit LYST:LYST-IP complex or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 function can be identified by use of known convenient *in vitro* assays, e.g., based on their ability to inhibit LYST:LYST-IP binding or as described *infra*.

In specific embodiments, Therapeutics that antagonize LYST:LYST-IP complex formation or activity, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 activity, are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an increased (relative to normal or desired) level of LYST:LYST-IP complex, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein.

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for example, in patients where a LYST:LYST-IP complex or said individual LYST-2 or novel LIP protein is overactive or overexpressed; (2) in diseases or disorders involving an absence or decreased (relative to normal or desired) level of said LYST:LYST-IP complex, or said individual LYST-2 or novel LIP protein, for example, in patients where LYST:LYST-IP complexes (or the individual components necessary to form the complexes), or where said individual LYST-2 or novel LIP protein is lacking, genetically defective, biologically inactive or underactive, or under-expressed; (3) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of a LYST:LYST-IP complex or individual LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 antagonist administration; or (4) in diseases or disorders wherein *in vitro* (or *in vivo*) assays (see *infra*) indicate the utility of LYST:LYST-IP complex, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 agonist administration.

Increased, decreased, or absent levels of LYST:LYST-IP complexes or of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein levels, can be readily detected, e.g., by quantifying protein and/or RNA, by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it in vitro for RNA or protein levels, structure and/or activity of the expressed LYST:LYST-IP complex (or for the concurrent expression of mRNA encoding the two components of the LYST:LYST-IP complex), or said individual LYST-2 or novel LIP protein or mRNA levels. Many methods standard in the art can be thus employed, including but not limited to: immunoassays to detect and/or visualize LYST:LYST-IP complexes, or LYST-2. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein (see, e.g., RECOMBINANT METHODOLOGIES supra, and ASSAYS sections. infra.) and/or hybridization assays to detect concurrent expression of LYST and a LYST-IP, or individual LYST-2 or novel LIP mRNA (e.g., Northern blot assays, dot blots, in situ hybridization, etc.).

## REDUCING LYST:LYST-IP COMPLEX EXPRESSION

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A more specific embodiment includes methods of reducing LYST:LYST-IP complex expression (*i.e.*, the expression of the two components of the LYST:LYST-IP complex and/or formation of the complex), or reducing LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7. LIP8, LIP9, or LIP10 expression, by targeting mRNAs that express the protein moieties. RNA therapeutics currently fall within three classes, antisense species, ribozymes, or RNA aptamers (Good *et al.*, 1997, *Gene Therapy 4*: 45-54).

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Antisense oligonucleotides have been the most widely used. By way of example, but not for limitation, antisense oligonucleotide methodology to reduce LYST:LYST-IP complex formation is presented below. Ribozyme therapy involves the administration, induced expression, etc., of small RNA molecules with enzymatic ability to cleave, bind, or otherwise inactivate specific RNAs to reduce or eliminate expression of particular proteins (Grassi and Marini, 1996, Annals of Medicine 28: 499-510, Gibson, 1996, Cancer and Metastasis Reviews 15: 287-299). At present, the design of hairpin and hammerhead RNA ribozymes is necessary to specifically target a particular mRNA, such as the mRNA encoding LYST or LYST-2. RNA aptamers are specific RNA ligands for proteins, such as for Tat and Rev RNA (Good et al., 1997, Gene Therapy 4: 45-54) that can specifically inhibit their translation. Aptamers specific for LYST or LYST-2 or a LYST-IP can be identified by many methods well known in the art, for example but not limited to the protein-protein interaction assay described in Section 5.7.1 infra.

In another embodiment, the activity or level of LYST or LYST-2 is reduced by administration of a LYST-IP, or a nucleic acid that encodes a LYST-IP, or antibody that immunospecifically binds to a LYST-IP, or a fragment or a derivative of the antibody containing the binding domain thereof. Additionally, the level or activity of a LYST-IP may be reduced by administration of a LYST or LYST-2 or a LYST-IP nucleic acid, or an antibody that immunospecifically binds LYST or LYST-2, or a fragment or derivative of the antibody containing the binding domain thereof.

In another aspect of the invention, diseases or disorders associated with increased levels of LYST or LYST-2 or a particular LYST-IP (e.g., see supra) may be treated or prevented by administration of a Therapeutic that increases LYST:LYST-IP complex formation, if the complex formation acts to reduce or inactivate LYST or LYST-2 or the particular LYST-IP through LYST:LYST-IP complex formation. Such diseases or disorders can be treated or prevented by administration of one member of the LYST:LYST-IP complex, including mutants of a member of the LYST:LYST-IP that have increased affinity for the other member of the LYST:LYST-IP complex (to cause increased complex formation), administration of antibodies or other molecules that stabilize the LYST:LYST-IP complex, etc.

## INCREASING LYST OR LYST-IP LEVELS

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In a specific embodiment, the activity or level of LYST or LYST-2 is increased by administration of a LYST-IP, or derivative or analog thereof, a nucleic acid encoding a LYST-IP, or an antibody that immunospecifically binds a LYST-IP, or a fragment or derivative of the

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antibody contains the binding domain thereof. In another specific embodiment, the activity or levels of a LYST-IP are increased by administration of LYST or LYST-2, or derivative or analog thereof, a nucleic acid encoding LYST or LYST-2, or an antibody that immunospecifically binds LYST or LYST-2, or a fragment or derivative of the antibody contains the binding domain thereof.

#### ORIGIN OF THE THERAPEUTIC

Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, a human LYST:LYST-IP complex, or LIP1. LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, or derivative or analog thereof, nucleic acids encoding the members of the human LYST:LYST-IP complex, or human LYST-2, human LIP1, human LIP2, human LIP3, human LIP4, human LIP5, human LIP6, human LIP7, human LIP8, and human LIP9, and human LIP10, or derivative or analog thereof, or an antibody to a human LYST:LYST-IP complex, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, or derivative thereof, is therapeutically or prophylactically administered to a human patient.

## DETERMINATION OF THE EFFECT OF THE THERAPEUTIC

Preferably, suitable *in vitro* or *in vivo* assays are utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of an affected tissue. In various specific embodiments, *in vitro* assays can be carried out with representative cells or cell types involved in a patient's disorder to determine if a Therapeutic has a desired effect upon such cell types.

Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. Additional descriptions and sources of Therapeutics that can be used according to the invention are found herein.

#### ATOPIC DISORDERS

LYST has been implicated in atopic disorders. Atopy is defined as high immunoglobulin E (IgE) responsiveness (Kawakami et al., 1997, Respirology 2: 7-15), since high serum IgE

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concentrations are found in individuals with atopic disorders. IgE plays a central role in mediating the pathology of allergic disease. In IgE allergic diseases both mast cells and T lymphocytes play an important role. Whereas mast cells have been implicated in immediate allergic responses, T-lymphocytes mediate subsequent late phase responses and chronic inflammation. Cellular mechanisms that mediate the sensitization, immediate and late-phase reactions that follow encounters with allergen, as well as cell surface and signaling events that result in mediator release from inflammatory cells have been discussed. One mechanism that has been postulated as cause for atopic disorders is the interaction of LYST protein with the IgE receptors on granular cells (mast cells) which causes the degranulation of these basophilic granulocytes and subsequently, the release of histamine. After emptying intracellular Ca2+ stores, basophils become extremely sensitive to stimuli that induce degranulation and extensive histamine release (Knol, 1996, Eur. Respir. J. Suppl. 22: 126S-131S). The interaction of LYST with the IgE receptor may be one cause for immunological disorders such as bronchial asthma, nasal polyps, hay fever (allergic) rhinitis, urticaria, atopic dermatitis, food allergies, nonhereditary angioedema, systemic anaphylaxis, and allergic conjunctivitis. All these atopic disorders are associated with a positive family history, and 20-30% of the population has a strong genetic predisposition for this condition (Casolaro et al., 1996, Curr. Opin. Immunol. 8: 796-803). Atopic dermatitis is the most common chronic skin disease of young children and is frequently associated with asthma and allergies (Boguniewicz, 1997, Curr. Opin. Pediatr. 9: 577-581).

Therapeutics of this invention, particularly those that modulate (or supply) LYST:LYST-IP activity may be effective in treating or preventing atopic diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of LYST:LYST-IP) can be assayed by any method known in the art for efficacy in treating or preventing such atopic diseases and disorders. Such assays include *in vivo* assays using animal models of atopic diseases or disorders, or *in vitro* assays as described *infra*. Potentially effective Therapeutics may act by decreasing the abnormal degranulation of basophilic granulocytes.

Accordingly, once an atopic disease or disorder has been shown to be amenable to treatment by modulation of LYST:LYST-IP complex activity, that atopic disease or disorder can be treated or prevented by administration of a Therapeutic that modulates the LYST:LYST-IP complex formation (including supplying LYST:LYST-IP complexes or function).

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#### **AUTOIMMUNE DISORDERS**

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LYST has been implicated in autoimmune disorders. Mice with LYST mutations (beige mice) do not develop renal failure when affected by systemic lupus erythematosus. LYST mutation appears to be protective from development of end-organ damage as a consequence of auto-immune disease (Clark et al., 1982, In: NK CELLS AND OTHER NATURAL EFFECTOR CELLS. Heberman, Ed., Academic Press, pp. 301-306). The LYST protein protects cells against autoimmune disease, including, but not limited to, arthritis (Barthold, 1995, J. Infect. Dis. 172: 778-784), systemic lupus, systemic autoimmune diseases, inflammatory bowel disease IBD. Crohn's disease, ulcerative colitis, organ specific autoimmune disease including thyroid disorders, diabetes mellitus and multiple sclerosis. It is known that the LYST interactant Imogen 38 is a target for autoimmune attack in diabetes mellitus. Thus, Therapeutics of the invention, particularly those that mimic, modulate (or supply) LYST:LYST-IP complex activity may be effective in treating or preventing autoimmune diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of LYST:LYST-IP) can be assayed by any method known in the art for efficacy in treating or preventing such autoimmune diseases and disorders. Such assays include in vitro assays using cell culture models as described infra, or in vivo assays using animal models of autoimmune diseases or disorders, also described infra. Potentially effective Therapeutics, for example but not by way of limitation, reduce autoimmune responses in animal models in comparison to controls.

Autoimmune disorders that can be treated or prevented include but are not limited to those listed in Isslebacher *et al.*, 1997 (In: HARRISON'S PRINCIPALS OF INTERNAL MEDICINE, 13<sup>th</sup> Ed., McGraw Hill, New York, pp. 1543-1559, incorporated herein by reference).

Accordingly, once an autoimmune disease or disorder has been shown to be amenable to treatment by modulation of LYST:LYST-IP complex activity, that autoimmune disease or disorder can be treated or prevented by administration of a Therapeutic that modulates the LYST:LYST-IP complex formation (including supplying LYST:LYST-IP complexes) or function.

#### **NEURODEGENERATIVE DISORDERS**

LYST and LYST-2 and certain binding partners of LYST and LYST-2 (Atrophin-I. DGS-I, 14-3-3 protein, HS1 protein, norbin, HBF-G2) have been implicated in neurodegenerative disease. The Chediak-Higashi syndrome is characterized by neurologic disorders such as peripheral neuropathy and ataxia. Beige mice show a progressive neurological disorder

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accompanied by a complete loss of cerebellar Purkinje cells (Murphy and Roths. 1978. Jackson Lab. Ann. Rep. 49: 108-109). Cerebellar and hippocampal cytoarchitechonic abnormalities are found in beige mutants (Guo et al.. 1992, Tokai J. Exp. Clin. Med. 17: 53-61). The LYST interactant Atrophin-I has been associated with the neurodegenerative disease dentatorubralpallidoluysian atrophy (DRPLA, Smith's disease). DGS-I is associated with a developmental disease DiGeorge syndrome. 14-3-3 protein which interacts with both LYST and LYST-2, is present in Alzheimer's disease neurofibrillary tangles and is present in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Furthermore, the LYST-2 interactant HBF-G3 is a human brain factor expressed in fetal tissues. LIP3, a homolog to rat Roaz protein, may have a role in regulation neuronal-specific gene expression. Further, LYST and certain binding partners of LYST (e.g., 14-3-3 protein, HS1 protein, calmodulin, casein kinase subunit II, Hrs. Efs, estrogen receptor related protein. norbinLIP1 (Tcp-10 homolog), LIP7 (Tcp-10 homolog), LIP5, LIP6 (Ns2-3 protease homolog), LIP4 (hnRNP-E2 homolog) and LIP8 (KAP4L)) have been implicated in signaling leading to exocytosis, degranulation, and vesicular protein trafficking. If defective, these mechanisms can lead to neurodegenerative disease.

Accordingly, Therapeutics of the invention, particularly but not limited to those that modulate (or supply) LYST:IP and complexes of LYST or LYST-2 and LYST-IPs may be effective in treating or preventing neurodegenerative disease. Therapeutics of the invention that modulate LYST:LYST-IP complexes involved in neurodegenerative disorders can be assayed by any method known in the art for efficacy in treating or preventing such neurodegenerative diseases and disorders. Such assays include *in vitro* assays for regulated cell secretion, protein trafficking, and/or folding or inhibition of apoptosis or *in vivo* assays using animal models of neurodegenerative and/or developmental diseases or disorders, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, promote regulated cell maturation and prevent cell apoptosis in culture, or reduce neurodegeneration in animal models in comparison to controls.

Such diseases include all degenerative disorders involved with aging, especially osteoarthritis and neurodegenerative disorders. Neurodegenerative disorders that can be treated or prevented include but are not limited to those listed in Isslebacher *et al.*, 1997 (In: HARRISON'S PRINCIPALS OF INTERNAL MEDICINE, 13<sup>th</sup> Ed., McGraw Hill, New York, incorporated herein by reference).

Once a neurodegenerative disease or disorder has been shown to be amenable to treatment by modulation of LYST:LYST-IP complex activity, that neurodegenerative disease or

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disorder can be treated or prevented by administration of a Therapeutic that modulates LYST:LYST-IP complex formation (including supplying LYST:LYST-IP complexes).

#### **ONCOGENESIS**

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#### **MALIGNANCIES**

Components of LYST:LYST-IP complexes (i.e., LYST and LYST-2, estrogen-receptor related protein, casein kinase II beta SU, 14-3-3 protein, FteI, BMK1, HS1protein, troponin) have been implicated in regulation of cell proliferation. The estrogen-receptor related protein has been found expressed in several cancers, including breast cancer, prostate cancer, pancreatic cancer, osteoblastic osteosarcoma, gastric cancer. laryngeal carcinoma, papillary thyroid carcinoma, intracranial tumors, colorectal cancer and colon cancer. 14-3-3 protein has been found in lung cancer cells, but also in other cancers, including colorectal adenocarcinoma, lymphoblastoma, salivary gland tumors. Chediak-Higashi patients have an increased tumor growth rate and higher metastatic frequency, and non-Hodgkin's lymphoma occurred in over one third of CHS patients (Hayakawa et al., 1986, Jpn. J. Cancer Res. 77: 74-79). This may be due to the involvement of natural killer cells in cancer.

Accordingly, Therapeutics of the invention may be useful in treating or preventing diseases or disorders associated with cell hyperproliferation or loss of control of cell proliferation, particularly cancers, malignancies and tumors. Therapeutics of the invention can be assayed by any method known in the art for efficacy in treating or preventing malignancies and related disorders. Such assays include *in vitro* assays using transformed cells or cells derived from the tumor of a patient, or *in vivo* assays using animal models of cancer or malignancies, or any of the assays described *infra*. Potentially effective Therapeutics, for example but not by way of limitation, inhibit proliferation of tumors or transformed cells in culture, or cause regression of tumors in animal models in comparison to controls. *e.g.*, as described *infra*. In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented in the bladder, breast, colon, lung, prostate, pancreas, or uterus.

Such cancers and malignancies include but are not limited to those listed in Fishman et al., 1985 MEDICINE. 2d Ed., J.B. Lippincott Co., Philadelphia, a review of such disorders (incorporated herein by reference).

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Accordingly, once a malignancy or cancer has been shown to be amenable to treatment by modulating (i.e., inhibiting, antagonizing, enhancing or agonizing) LYST:LYST-IP complex activity, or modulating LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, activity, that cancer or malignancy can be treated or prevented by administration of a Therapeutic that modulates LYST:LYST-IP complex formation and function, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 function, including supplying LYST:LYST-IP complexes and the individual binding partners of a LYST:LYST-IP complex.

## PREMALIGNANT CONDITIONS

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The Therapeutics of the invention that are effective in treating cancer or malignancies (e.g., as described above) can also be administered to treat premalignant conditions and to prevent progression to a neoplastic or malignant state. Such prophylactic or therapeutic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79). Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult cell or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, skin, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention that modulates LYST:LYST-IP complex activity, or that modulates LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 activity.

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Such characteristics of a transformed phenotype include morphological changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250 kDa cell surface protein, etc. (see also Id., pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention. In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention. In another specific embodiment, a Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, lung, pancreatic, prostate or uterine cancer, or melanoma or sarcoma.

In other embodiments, a patient that exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (e.g., the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (e.g., hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, neurofibromatosis of Von Recklinghausen, retinoblastoma, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; see Robbins and Angell. 1976, BASIC PATHOLOGY, 2nd Ed., W.B. Saunders Co., Philadelphia, pp. 112-113, etc., incorporated herein by reference).

## HYPERPROLIFERATIVE AND DYSPROLIFERATIVE DISORDERS

In another embodiment of the invention, a Therapeutic is administered to treat or prevent hyperproliferative or benign dysproliferative disorders. Therapeutics of the invention can be assayed by any method known in the art for efficacy in treating or preventing hyperproliferative diseases or disorders, such assays include *in vitro* cell proliferation assays. *in vitro* or *in vivo* assays using animal models of hyperproliferative diseases or disorders, or any of the assays

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described infra. Potentially effective Therapeutics include but are not limited to. Therapeutics that reduce cell proliferation in culture or inhibit growth or cell proliferation in animal models in comparison to controls.

Accordingly, once a hyperproliferative disorder has been shown to be amenable to treatment by modulation of LYST:LYST-IP complex activity, or by modulation of LYST-2. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein activity, that hyperproliferative disease or disorder can be treated or prevented by administration of a Therapeutic that modulates LYST:LYST-IP complex formation, or that modulates LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 activity (including supplying a LYST:LYST-IP complex and/or the individual binding partners of a LYST:LYST-IP complex).

Specific embodiments are directed to treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), benign tumors, fibrocystic conditions, and tissue hypertrophy (e.g., prostatic hyperplasia).

## PIGMENTATION DISORDERS

LYST is strongly implicated in pigmentation disorders, such as oculocutaneous albinism and hypopigmentation. Albinism connotes a large group of genetic disorders that are characterized by diminished ocular and often cutaneous pigmentation. Genetically based syndromes such as Chediak-Higashi syndrome (CHS), phenotypically resembling albinism, in which the synthesis of pigmented melanosomes, as well as specialized organelles of other cell types, is compromised. Oculocutaneous albinism is characterized by a congenital reduction or absence of melanin pigment in the skin, hair and eyes. The reduction in the hair and skin results in a change in color but no change in the development or function of these tissues, while the absence of melanin pigment in the eye leads to abnormal development and function.

Therapeutics of the invention, particularly those that modulate (or supply) LYST:LYST-IP complex activity may be effective in treating or preventing pigmentation diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of LYST:LYST-IP complexes) can be assayed by any method known in the art for efficacy in treating or preventing such viral diseases and disorders. Such assays include widely used in vitro assays using cell culture models, and in vivo assays using animal models of pigmentation

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diseases or disorders (see McGeoch et al., 1986, J. Gen. Virol. 67: 813-825 for review). Potentially effective Therapeutics, for example but not by way of limitation, reduce pigmentation diseases or disorders in animal models in comparison to controls.

Accordingly, once a pigmentation disease or disorder has been shown to be amenable to treatment by modulation of LYST:LYST-IP complex activity, that pigmentation disease or disorder can be treated or prevented by administration of a Therapeutic that modulates LYST:LYST-IP complex formation, including supplying a LYST:LYST-IP complex, or LYST-2 or at least one of the ten novel LIP genes or proteins.

PLATELET DYSFUNCTION

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LYST is strongly implicated in platelet abnormality that can lead to increased bleeding tendency in Chediak-Higashi syndrome. Dense bodies of platelets, lysosomes of leukocytes and fibroblasts, azurophilic granules of neutrophils and melanosomes of melanocytes are generally larger in size and irregular in morphology in CHS patients, indicating that a common pathway in storage organellogenesis is affected in those patients. Platlet dysfunction that may be treated by modulation of LYST:LYST-IP complex activity is of two general classes: (1) diseases associated with platelet storage pool deficiency (e.g., Hermansky-Pudlak Syndrome, Chediak-Higashi Syndrome, gray platelet syndrome) or thrombocytopenia; and (2) diseases associated with thrombocytosis or increased clotting tendency (e.g., myocardial infarction, deep venous thrombosis, cardiovascular accident, transient ischemic attack). The platelet storage pool deficiency in CHS is associated with prolonged bleeding time and low dense granule content (Novak et al., 1984, Blood 63: 536-544).

Therapeutics of the invention, particularly those that modulate (or supply) LYST:LYST-IP complex activity may be effective in treating or preventing platelet dysfunction diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of LYST:LYST-IP complexes) can be assayed by any method known in the art for efficacy in treating or preventing such platelet dysfunction diseases and disorders. Such assays include widely used *in vitro* assays using cell culture models, and *in vivo* assays using animal models of platelet dysfunction diseases or disorders (see McGeoch *et al.*, 1986, *J. Gen. Virol.* 67: 813-825 for review). Potentially effective Therapeutics, for example but not by way of limitation, reduce platelet dysfunction diseases or disorders in animal models in comparison to controls.

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Accordingly, once a platelet dysfunction disease or disorder has been shown to be amenable to treatment by modulation of LYST:LYST-IP complex activity, that platelet dysfunction disease or disorder can be treated or prevented by administration of a Therapeutic that modulates LYST:LYST-IP complex formation, including supplying a LYST:LYST-IP complex.

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#### VIRAL INFECTION

LYST and the LYST interactant Importin beta subunit are strongly implicated in viral infection mechanisms, including that for the AIDS virus, HIV-1. An enormous number of human diseases result from virulent and opportunistic viral infection (see, e.g., Belshe (Ed.) 1984 TEXTBOOK OF HUMAN VIROLOGY, PSG Publishing, Littleton, MA). Viral diseases of a wide array of tissues, including the respiratory tract, the CNS, the skin, the genitourinary tract, the eyes and ears, the immune system, the gastrointestinal tract, and the musculoskeletal system, affect a vast number of humans, of all ages (see Table 328-2, In: Wyngaarden and Smith, 1988, CECIL TEXTBOOK OF MEDICINE, 18th Ed., W.B. Saunders Co., Philadelphia, pp.1750-1753).

In particular embodiments, in diseases and disorders including, but not limited to, e.g. those diseases and disorders described herein, Therapeutics of the invention, particularly those that modulate (or supply) LYST:LYST-IP complex (particularly LYST:importin beta subunit complex) activity may be effective in treating or preventing viral diseases or disorders. Therapeutics of the invention (particularly Therapeutics that modulate the levels or activity of LYST:importin beta subunit complex) can be assayed by any method known in the art for efficacy in treating or preventing such viral diseases and disorders. Such assays include widely used in vitro assays using cell culture models. and in vivo assays using animal models of viral diseases or disorders (see McGeoch et al., 1986, J. Gen. Virol. 67: 813-825 for review). Potentially effective Therapeutics, for example but not by way of limitation, reduce viral responses in animal models in comparison to controls.

Accordingly, once a viral disease or disorder has been shown to be amenable to treatment by modulation of Importin beta subunit complex activity, that viral disease or disorder can be treated or prevented by administration of a Therapeutic that modulates LYST:LYST-IP complex (particularly LYST:Importin beta subunit complex) formation. including supplying a LYST:LYST-IP complex, particularly a LYST:Importin beta subunit complex).

#### 67 GENE THERAPY

In a specific embodiment, a nucleic acid molecule comprising a sequence encoding LYST or LYST-2 and/or a LYST-IP, or an individual LYST-2 or novel LIP protein, or a functional derivative thereof, are administered to modulate LYST:LYST-IP complexes, or to modulate LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 function, by way of gene therapy. In more specific embodiments, at least one nucleic acid encoding either or both LYST or LYST-2 and a LYST-IP, or functional derivatives thereof, are administered by way of gene therapy. Gene therapy refers to therapy performed by the administration of a nucleic acid molecule to a subject. In this embodiment of the invention, the nucleic acid molecule produces its encoded protein(s) that mediates a therapeutic effect by modulating the LYST:LYST-IP complex, or by modulating LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 function. Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

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For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12: 488-505; Wu and Wu, 1991, Biotherapy 3: 87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32: 573-596; Mulligan, 1993, Science 260: 926-932; Morgan and Anderson, 1993, Ann. Rev. Biochem. 62: 191-217; and May, 1993, TIBTECH 11: 155-215). Methods commonly known in the art for recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a preferred aspect, the Therapeutic comprises a LYST or LYST-2 and/or a LYST-IP nucleic acid, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6. LIP7, LIP8, LIP9, or LIP10 nucleic acid, that is part of an expression vector that expresses the LYST or LYST-IP protein(s), or expresses a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein. or fragment or a chimeric protein thereof, in a suitable host. In particular, such a nucleic acid has a promoter(s) operably linked to the LYST and/or the LYST-IP coding region(s), or linked to the LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 coding region, said promoter(s) being inducible or constitutive, and optionally, tissue-specific. See, e.g., as discussed. supra, in RECOMBINANT METHODOLOGIES. In another particular embodiment, a nucleic acid molecule is used in which the LYST or LYST-2 and/or LYST-IP coding sequence. or the individual LYST-2 or novel LIP coding sequences, and any other desired sequences, are flanked by regions that promote homologous recombination at a desired site in the genome, thus

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providing for intra-chromosomal expression of the LYST and the LYST-IP nucleic acids (Koller and Smithies, 1989, *Proc. Natl. Acad. Sci. USA 86*: 8932-8935, Zijlstra *et al.*, 1989, *Nature* 342: 435-438).

Delivery of the nucleic acid into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vector, or indirect, in which case, cells are first transformed with the nucleic acid *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* and ex vivo gene therapy.

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In a specific embodiment, the nucleic acid is directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by infection using a defective or attenuated retroviral or other viral vector (see, U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by encapsulation in liposomes, microparticles, or microcapsules, or by administering it in linkage to a peptide which is known to enter the nucleus, or by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262: 4429-4432), which can be used to target cell types specifically expressing the receptors, etc. In another embodiment, a nucleic acid-ligand complex can be formed in which the ligand comprises a fusogenic viral peptide that disrupts endosomes, preventing lysosomal degradation of the nucleic acid. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression by targeting a specific receptor (see. e.g., International Patent Publications WO 92/06180, WO 92/22635, WO 92/20316, WO 93/14188, and WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86: 8932-8935, Zijlstra et al., 1989, Nature 342: 435-438).

In a specific embodiment, a viral vector that contains the LYST or LYST-2 and/or the LYST-IP encoding nucleic acid sequence, or the individual LYST-2 or novel LIP encoding nucleic acid sequence, is used. For example, a retroviral vector can be used (see Miller et al., 1993, Meth. Enzymol. 217: 581-599). These retroviral vectors have been modified to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The LYST or LYST-2 and/or LYST-IP (preferably both LYST and LYST-IP) encoding nucleic acids, or individual LYST-2 or novel LIP encoding nucleic acids, to be used in

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gene therapy is/are cloned into the vector, which facilitates delivery of the gene into a patient. More detail about retroviral vectors and the use of retroviral vectors in gene therapy can be found in Boesen et al., 1994, Biotherapy 6: 291-302, Clowes et al., 1994, J. Clin. Invest. 93: 644-651, Kiem et al., 1994, Blood 83: 1467-1473, Salmons et al., 1993, Human Gene Therapy 4: 129-141, and Grossman et al., 1993, Curr. Opin. Genetics Devel. 3: 110-114.

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson. 1993, Current Opinion in Genetics and Development 3: 499-503 present a review of adenovirus-based gene therapy. Bout et al., 1994, Human Gene Therapy 5: 3-10. demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, Science 252: 431-434, Rosenfeld et al., 1992, Cell 68: 143-155, and Mastrangeli et al., 1993, J. Clin. Invest. 91: 225-234. Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, Proc. Soc. Exp. Biol. Med. 204: 289-300).

Another approach to gene therapy involves transferring a gene into cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that took up and are expressing the transferred gene. Those cells are then delivered to a patient by various means available in the art.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr. 1993, Meth. Enzymol. 217: 599-618, Cohen et al., 1993, Meth. Enzymol. 217: 618-644, Cline, 1985, Pharmac. Ther. 29: 69-92) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the

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nucleic acid to the cell, so that the nucleic acid is expressible by the cell, and is heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. In a preferred embodiment, epithelial cells are injected, e.g., subcutaneously. In another embodiment, recombinant skin cells may be applied as a skin graft onto the patient. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. Recombinant secretory cells (e.g., cells modified to express dopamine) may be encapsulated and implanted in a host by various methods known in the art. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes, nucleated blood cells, various stem or progenitor cells, in particular embryonic heart muscle cells, liver stem cells (International Patent Publication WO 94/08598), neural stem cells (Stemple and Anderson, 1992, Cell 71: 973-985), hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc. In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

Epithelial stem cells (ESCs) or keratinocytes can be obtained from tissues such as the skin and the lining of the gut by known procedures (Rheinwald, 1980. *Meth. Cell Bio. 21*: 229). ESCs or keratinocytes obtained from the skin or lining of the gut of a patient or donor can be grown in tissue culture (Rheinwald, 1980, *Meth. Cell Bio. 21*α: 229; Pittelkow and Scott, 1986, *Mayo Clinic Proc. 61*: 771). If the ESCs are provided by a donor, a method for suppression of host versus graft reactivity (*e.g.*, irradiation, drug or antibody administration to promote moderate immunosuppression) can also be used.

With respect to hematopoietic stem cells (HSC), any technique which provides for the isolation, propagation, and maintenance *in vitro* of HSCs can be used in this embodiment of the invention. Techniques by which this may be accomplished include (a) the isolation and establishment of HSC cultures from bone marrow cells isolated from the future host, or a donor, or (b) the use of previously established long-term HSC cultures, which may be allogeneic or xenogeneic. Non-autologous HSC are used preferably in conjunction with a method of suppressing transplantation immune reactions of the future host/patient. In a particular embodiment of the present invention, human bone marrow cells can be obtained from the

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posterior iliac crest by needle aspiration (see. e.g., Kodo et al., 1984, J. Clin. Invest. 73: 1377-1384). In a preferred embodiment of the present invention, the HSCs can be made highly enriched or in substantially pure form. This enrichment can be accomplished before, during, or after long-term culturing, and can be done by any technique known in the art. Long-term cultures of bone marrow cells can be established and maintained by using, for example, modified Dexter cell culture techniques (Dexter et al., 1977, J. Cell Physiol. 91: 335) or Witlock-Witte culture techniques (Witlock and Witte, 1982, Proc. Natl. Acad. Sci. USA 79: 3608-3612).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Additional methods can be adapted for use to deliver a nucleic acid molecule encoding the LYST or LYST-2 and/or LYST-IP proteins. or functional derivatives thereof. e.g., as described supra.

# USE OF ANTISENSE OLIGONUCLEOTIDES FOR SUPPRESSION OF LYST:LYST-IP COMPLEXES OR FOR SUPPRESSION OF LYST-2 OR NOVEL LIP PROTEIN EXPRESSION

In a specific embodiment, LYST:LYST-IP complex function or individual LYST-2 or novel LIP protein function is inhibited by use of antisense nucleic acids for LYST or LYST-2 and/or a LYST-IP, (preferably both LYST and the LYST-IP), or individual antisense nucleic acids for LYST-2. LIP1. LIP2, LIP3, LIP4, LIP5. LIP6. LIP7, LIP8, LIP9, or LIP10. The present invention provides the therapeutic or prophylactic use of nucleic acids of at least 6 nucleotides that are antisense to a gene or cDNA encoding LYST or LYST-2 and/or a LYST-IP, or encoding LYST-2, LIP1, LIP2, LIP3, LIP4. LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, or a portion thereof. A LYST or a LYST-IP "antisense" nucleic acid as used herein refers to a nucleic acid capable of hybridizing to a portion of LYST or a LYST-IP nucleic acid (preferably mRNA) by virtue of some sequence complementarity. The antisense nucleic acid may be complementary to a coding and/or noncoding region of a LYST or LYST-IP mRNA. Such antisense nucleic acids have utility as Therapeutics that inhibit LYST:LYST-IP complex formation or activity, or individual LYST-2 or novel LIP protein function or activity, and can be used in the treatment or prevention of disorders as described, *supra*.

The antisense nucleic acids of the invention can be oligonucleotides that are doublestranded or single-stranded. RNA or DNA or a modification or derivative thereof, which can be

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directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

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In another embodiment, the invention is directed to methods for inhibiting the expression of LYST and/or a LYST-IP nucleotide sequence, or individual LIP1, LIP2, LIP3, LIP4, LIP5. LIP6, LIP7, LIP8, LIP9, or LIP10 nucleotide sequences, in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense nucleic acid of LYST or LYST-2 and LYST-IP, or an antisense nucleic acid of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, or a derivative thereof, of the invention, as described in REDUCING LYST:LYST-IP COMPLEX EXPRESSION, *supra*.

The LYST or LYST-2 and/or LYST-IP antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 200 oligonucleotides). In specific aspects, the oligonucleotide is at least about 10 nucleotides, at least about 15 nucleotides, at least about 100 nucleotides, or at least about 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86: 6553-6556, Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84: 648-652, PCT Publication No. WO 88/09810) transport across the bloodbrain barrier (see, e.g., PCT Publication No. WO 89/10134), hybridization-triggered cleavage agents (see, e.g., Krol et al., 1988, BioTechniques 6: 958-976), or intercalation with other agents (see, e.g., Zon, 1988, Pharm. Res. 5: 539-549).

In a preferred aspect of the invention, a LYST or LYST-2 and/or LYST-IP antisense oligonucleotide is provided, preferably as single-stranded DNA. The oligonucleotide may be modified at any position on its structure with constituents generally known in the art.

The LYST or LYST-2 and/or LYST-IP antisense oligonucleotides may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 5-methylguanine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5N-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5N-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5N-

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methoxycarboxymethyluracil. 5-methoxyuracil. 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine. 5-methyl-2-thiouracil. 2-thiouracil. 4-thiouracil. 5-methyluracil. uracil-5-oxyacetic acid methylester. uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil. 3-(3-amino-3-N-2-carboxypropyl) uracil. (acp3)w, and 2.6-diaminopurine.

In a particular embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose. In another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphorodithioate, a phosphoramidate, a phosphoramidate, a methylphosphonate, an alkyl phosphotriester, or a formacetal or analog thereof. In yet another embodiment, the oligonucleotide is a 2-anomeric oligonucleotide. An anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gautier *et al.*, 1987, *Nucl. Acids Res. 15*: 6625-6641).

Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al., 1988, Nucl. Acids Res. 16: 3209, methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85: 7448-7451), etc.

In a specific embodiment, the LYST or LYST-2 and/or LYST-IP antisense oligonucleotides comprise catalytic RNAs, or ribozymes (see, e.g., PCT International Publication WO 90/11364, published October 4, 1990, Sarver et al., 1990, Science 247: 1222-1225). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15: 6131-6148), or a chimeric RNA-DNA analog (Inoue et al., 1987, FEBS Lett. 215: 327-330). In yet another embodiment, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent. hybridization-triggered cleavage agent, etc.

In an alternative embodiment, the LYST and/or LYST-IP antisense nucleic acids of the invention are produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the

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invention. Such a vector would contain a sequence encoding LYST or LYST-2 and/or a LYST-IP (preferably, both a LYST or LYST-2 and a LYST-IP antisense nucleic acid) antisense nucleic acid(s), or individual LYST-2. LIP1. LIP2, LIP3, LIP4. LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated. as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art to be capable of replication and expression in mammalian cells. Expression of the sequences encoding the LYST or LYST-2 and/or LYST-IP antisense RNAs can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. See description in RECOMBINANT TECHNOLOGIES section, *supra*.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a LYST or LYST-2 or a LYST-IP gene, preferably a human LYST or LYST-2 or LYST-IP gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded LYST or LYST-2 or LYST-IP antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex nucleic acid formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid. the more base mismatches with a LYST or LYST-2 or LYST-IP RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

In a preferred embodiment, single-stranded DNA antisense LYST and LYST-IP oligonucleotides, or single-stranded DNA antisense to the same, or individual LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 antisense oligonucleotides, or single-stranded DNA antisense to the same, is used.

Cell types that express or overexpress LYST or LYST-2 and/or LYST-IP mRNA, or individual LYST-2 or novel LIP RNA can be identified by various methods known in the art. Such methods include, but are not limited to, those methods detailed in the RECOMBINANT METHODOLOGIES section, *supra*, or the various ASSAYS sections, *infra*. In a preferred aspect.

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primary tissue from a patient can be assayed for LYST or LYST-2 and/or LYST-IP expression prior to treatment, e.g., by immunocytochemistry or in situ hybridization.

Pharmaceutical compositions of the invention (see *infra*), comprising an effective amount of a LYST or LYST-2 and/or a LYST-IP antisense nucleic acid in a pharmaceutically acceptable carrier, can be administered to a patient having a disease or disorder that is of a type that expresses or overexpresses LYST:LYST-IP complexes, LYST and/or LYST-IP mRNA, or LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 mRNA or protein.

The amount of LYST or LYST-2 and/or LYST-IP antisense nucleic acid that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity *in vitro*, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising LYST or LYST-2 or LYST-IP antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the LYST or LYST-2 and/or LYST-IP antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable central nervous system cell types (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87: 2448-2451, Renneisen et al., 1990, J. Biol. Chem. 265: 16337-16342).

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## FUNCTIONAL ASSAYS OF LYST:LYST-IP COMPLEXES, AND LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, LIP10 PROTEINS

The functional activity of a LYST:LYST-IP complex, or the functional activity of a LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, and derivatives, fragments and analogs thereof, can be assayed by various methods known in the art. Potential modulators (e.g., inhibitors, agonists and antagonists) of LYST:LYST-IP complex activity, or of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 activity (e.g., antibodies specific to LYST-2 or to an individual novel LIP, and LYST or LYST-2 or LYST-IP antisense nucleic acids) can be assayed for the ability to modulate LYST:LYST-IP complex formation and/or activity, and for the ability to modulate LYST-2 or individual novel LIP activity.

#### **IMMUNOASSAYS**

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Immunoassays which can be used include but are not limited to competitive and noncompetitive assay systems. For example, in one embodiment, where one is assaying for the ability to bind or compete with wild-type LYST:LYST-IP complexes, or LYST-2. LIP1. LIP2. LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, for binding to anti-LYST:LYST-IP antibodies, or antibodies specific to LYST-2 or any one of the ten novel LIP proteins, various immunoassays known in the art can be used, including but not limited to competitive and noncompetitive assay systems using techniques such as radioimmunoassays, enzyme linked immunosorbent assay (ELISA), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, in situ immunohistochemistry assays (using colloidal gold, enzyme or radioisotope labels, for example), Western blots, immunoprecipitation assays, precipitin reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A immunoassays, immunoelectrophoresis assays, etc. In one embodiment, antibody binding is assayed by detecting a label on the primary antibody. In another embodiment, the primary antibody is assayed by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. See, e.g., Coligan et al., CURRENT PROTOCOLS IN IMMUNOLOGY, R. Coico (Ed.), John Wiley & Sons, New York, 1998.

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#### **BINDING ASSAYS**

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Derivatives (e.g., fragments) and analogs of LYST-IPs can be assayed for binding to LYST or LYST-2 by any method known in the art, for example the modified yeast two hybrid assay system described *infra*, immunoprecipitation with an antibody that binds to LYST or LYST-2 in a complex followed by analysis by size fractionation of the immunoprecipitated proteins (e.g., by denaturing or nondenaturing polyacrylamide gel electrophoresis), Western analysis, non-denaturing gel electrophoresis, etc.

#### ASSAYS FOR GENE EXPRESSION

The expression of the LYST or LYST-2 and/or LYST-IP genes (both endogenous genes and those expressed from recombinant DNA containing these genes) can be detected using techniques known in the art, including but not limited to Southern hybridization, Northern hybridization, restriction endonuclease mapping, DNA sequence analysis, polymerase chain reaction amplification (PCR), or RNase protection with probes specific for LYST or LYST-2 or LYST-IP genes, in various cell types. See, e.g., Ausubel, et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, New York, 1997; Sambrook et al., 1989, MOLECULAR CLONING, A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, New York. Methods of amplification other than PCR commonly known in the art can be employed.

In one embodiment, Southern hybridization can be used to detect genetic linkage of LYST or LYST-2 or LYST-IP gene mutations to physiological or pathological states. Various cell types, at various stages of development or from patients at various disease stages, can be characterized for their expression of LYST or LYST-2 and/or a LYST-IP (particularly expression of LYST or LYST-2 and/or a LYST-IP at the same time and in the same cells), or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein expression. The stringency of the hybridization conditions for northern or Southern blot analysis can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific probes used, as described *supra*. Modifications to these methods and other methods commonly known in the art can be used.

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#### ASSAYS FOR BIOLOGICAL ACTIVITY

One embodiment of the invention provides a method for screening a derivative or analog of LYST or LYST-2 for biological activity comprising contacting said derivative or analog of

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LYST or LYST-2 with a protein selected from the group consisting of 14-3-3 protein. HS1 protein, Hrs. BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, ERRa1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 and detecting the formation of a complex between said derivative or analog of LYST or LYST-2 and said LYST-IP protein; wherein detecting formation of said complex indicates that said derivative or analog of LYST or LYST-2 has biological (e.g., binding) activity. Additionally, another embodiment of the invention relates to a method for screening a derivative or analog of a protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, ERRa1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 for biological activity comprising contacting said derivative or analog of said protein with LYST or LYST-2; and detecting the formation of a complex between said derivative or analog of said protein and LYST or LYST-2; wherein detecting the formation of said complex indicates that said derivative or analog of said protein has biological activity.

#### METHODS OF MODULATING THE PROTEIN ACTIVITY

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The present invention also provides methods of modulating the activity of a protein that can participate in a LYST:LYST-IP complex by administration of a binding partner of that protein, or derivative or analog thereof. LYST or LYST-2, and derivatives and analogs thereof, can be assayed for the ability to modulate the activity or level of a LYST-IP by contacting a cell, or administering to an animal, expressing a LYST-IP gene with a LYST or LYST-2 protein, or a nucleic acid encoding a LYST or LYST-2 protein, or an antibody that immunospecifically binds the LYST or LYST-2 protein, or a fragment or derivative of said antibody containing the binding domain thereof, and measuring a change in LYST-IP levels or activity, wherein a change in LYST-IP levels or activity indicates that LYST or LYST-2 can modulate LYST-IP levels or activity. Alternatively, a LYST-IP can be assayed for the ability to modulate the activity or levels of a LYST or LYST-2 protein by contacting a cell, or administering to an animal. expressing a LYST or LYST-2 gene with a LYST-IP, or a nucleic acid encoding a LYST-IP, or an antibody that immunospecifically binds to a LYST-IP, or a fragment or derivative of said antibody containing the binding domain thereof, wherein a change in LYST or LYST-2 levels or activity indicates that the LYST-IP can modulate LYST or LYST-2 levels or activity indicates that the LYST-IP can modulate LYST or LYST-2 levels or activity indicates that the LYST-IP can modulate LYST or LYST-2 levels or activity.

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The LYST:LYST-IP complex, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, or derivative, analog, or fragment thereof, can also be screened for activity in modulating (i.e., increasing or decreasing) the activity of LYST and the LYST binding partners (i.e., the LYST-IPs involved in particular LYST:LYST-IP complexes). Specific LYST:LYST-IP complexes may be associated with specific diseases or tissue types. The relevance of different LYST-IP proteins in specific diseases or disorders are set forth in the background section.

#### ASSAYS FOR TREATMENT OF ATOPIC DISORDERS

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LYST protein has been implicated in atopic disorders (see *supra*), including asthma and allergic diseases. Accordingly, LYST:LYST-IP complexes, and derivatives, analogs, and fragments thereof, nucleic acids encoding the LYST genes, anti-LYST:LYST-IP antibodies and antisense nucleic acids, and other modulators of the LYST:LYST-IP complex, can be tested for activity in treating or preventing atopic disorders in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing atopic disorders by contacting cultured cells that exhibit an indicator of an atopic reaction, *in vitro* with the Therapeutic, and comparing the level of indicator in the cells contacted with the Therapeutic with said level of the indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing atopic disorders. Cell models that can be used for such assays include, but are not limited to a model of EBV-B cells for serum-IgE-facilitated allergen presentation in atopic disease (van der Heijden *et al.*, 1993, *J. Immunol. 150*:3643-3650), *in vitro* cell culture model for allergic rhinitis of human nasal epithelial cell membrane (Varsano *et al.*, 1996, *Laryngoscope 106*:599-604).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing atopic disease by administering said Therapeutic to a test animal exhibiting an atopic reaction or which test animal does not exhibit an atopic reaction and is subsequently challenged with an agent that elicits an atopic reaction; and measuring the change in the atopic reaction after the administration of said Therapeutic, wherein a reduction in said atopic reaction or a prevention of said atopic reaction indicates that the Therapeutic has activity in treating or preventing an atopic disease.

A number of animal models of atopic disorders are known in the art. These models accurately mimic natural human atopic diseases. Examples of specific models include, but are not limited to, a mouse model of atopic asthma where anti-interleukin-4 inhibits IgE production

(Zhou et al., 1997, J. Asthma 34: 195-201); a primate model of asthma using anti-allergy/anti-asthma drugs (Turner et al., 1996, Inflamm. Res. 45: 239-245); a mouse model of allergen-induced airway inflammation and epithelial changes (Blyth et al., 1996, Am. J. Respir. Cell Mol. Biol. 14: 425-438); model of experimental allergic rhinitis using Japanese cedar pollen in guinea pigs (Nabe et al., 1997, Jpn. J. Pharmacol. 1997 75: 243-251); trimellitic anhydride-sensitive mouse as an animal model for urticaria (Lauerma et al., 1997, J. Appl. Toxicol. 17: 357-360); guinea pig ear as a model for testing non-immunologic contact urticants (Ng, 1988, Ann. Acad. Med. Singapore 17: 563-568); a patch test reaction to study the pathogenesis of atopic dermatitis (Bruijnzeel et al., 1993, Clin. Exp. Allergy 23: 97-109); a murine model of systemic and local anaphylaxis (Shin et al., 1997, Pharmacol. Res. 36: 141-146); a guinea pig model of conjunctival allergic reaction (Meijer et al., 1996, Prostaglandins 1996, 52: 431-446); and a murine model of allergic conjunctivitis (Merayo-Lloves et al., 1996, J. Allergy Clin. Immunol. 97: 1129-1140).

#### ASSAYS FOR TREATMENT OF AUTOIMMUNE DISEASES

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LYST and particularly the binding partner Imogen 38 have been implicated in autoimmune disease. Accordingly, LYST:LYST-IP complexes, particularly LYST:Imogen 38 complexes, and derivatives, analogs, and fragments thereof, nucleic acids encoding the LYST and Imogen 38 genes, anti-LYST:LYST-IP and particularly anti-LYST:Imogen 38 antibodies and antisense nucleic acids, and other modulators of the LYST:LYST-IP and particularly LYST:Imogen 38 complex activity, can be tested for activity in treating or preventing autoimmune disease in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the present invention can be assayed for activity in treating or preventing autoimmune disease by contacting cultured cells that exhibit an indicator of an autoimmune reaction, e.g., chemokine secretion, in vitro with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing autoimmune disease. Cell models that can be used for such assays include, but are not limited to, leukocyte and other synovial cells that secrete chemokines mediating inflammation (Kunkel et al., 1996, J. Leukoc. Biol. 59: 6-12), cerebrospinal fluid cells from animal models of multiple sclerosis (Norga et al., 1995, Inflamm. Res. 44: 529-534), macrophages in experimental autoimmunoneuritis, a model of Guillain-Barre Disease (Bai et al., 1997, J. Neuroimmunol. 76: 177-184), expression of CD40/CD40L in monocytes (Laman et al., 1996, Crit. Rev. Immunol. 16: 59-108), lymphocyte cultures from lpr

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mice (Nagata. 1996. *Prog. Mol. Subcell. Biol. 16*: 87-103), and cultured thyrocytes in spontaneous murine autoimmune thyroiditis (Green *et al.*. 1996, *Endocrinology 137*: 2823-2832).

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In another embodiment, a Therapeutic of the present invention can be assayed for activity in treating or preventing autoimmune disease by administering said Therapeutic to a test animal exhibiting an autoimmune reaction or which test animal does not exhibit an autoimmune reaction and is subsequently challenged with an agent that elicits an autoimmune reaction; and measuring the change in the autoimmune reaction after the administration of said Therapeutic, wherein a reduction in said autoimmune reaction or a prevention of said autoimmune reaction indicates that the Therapeutic has activity in treating or preventing an autoimmune disease.

A number of animal models of autoimmune disease are known in the art. These models, including those for arthritis, systemic lupus erythematosus, diabetes, etc., accurately mimic natural human autoimmune diseases (Farine, 97, Toxicol. 119: 29-35) and include, but are not limited to, experimental allergic encephalomyelitis for multiple sclerosis (Brabb et al., 1997, J. Immunol. 159: 497-507), thyroglobulin-induced experimental thyroiditis, multiple organ-localized autoimmune disease, e.g., thyroiditis and gastritis in BALB/c nu/nu mice receiving rat thymus grafts under their renal capsules (Taguchi and Takahashi, 1996, Immunology 89: 13-19), virus-induced autoimmune diseases such as insulin-dependent diabetes mellitus (Oldstone and von Herath. 1996 APMIS. 104: 689-97. Review), experimental autoimmune encephalomyelitis (Encinas et al.. 1996, J. Neurosci. Res. 45: 655-669). experimental autoimmune labyrinthitis (Gloddek et al.. 1994, Clin. Exp. Immunol. 97: 133-137), Freund's-adjuvant induced rheumatoid arthritis (Zahiri et al.. 1969, Can. Med. Assoc. J. 101: 259-278), inbred mouse strains that develop systemic lupus erythematosus, rheumatoid arthritis, graft-vs-host disease, and diabetes (Humphreys-Beher, 1996, Adv. Dent. Res. 10: 73-75), and autoimmune hepatitis (Meyer zum Buschenfelde and Dienes, 1996, Virchows Arch. 429: 1-12).

#### ASSAYS FOR TREATMENT OF NEURODEGENERATION DISORDERS

LYST and LYST-2, and the LYST and LYST-2 binding partners, such as 14-3-3 protein. HS1 protein. Atrophin-I. GBDR1, HBF-G2, and DGS-I. have been found in brain tissue (see also Sections 2, 5.5.1 and 5.5.8). It is likely that the LYST interactants LIP3 (roaz homolog) and norbin are also located in brain tissue. Affected individuals with the Chediak-Higashi syndrome have, among other symptoms, neurologic disorders such as peripheral neuropathy and ataxia. In the case of Atrophin-I. expression of a protein has been genetically-linked to neurodegenerative

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disease, the dentatorubral pallidoluysian atrophy (DRPLA, Smith's disease). 14-3-3 protein is present both in Alzheimer's disease neurofibrillary tangles and in the cerebrospinal fluid of Creutzfeldt-Jakob disease patients. HBF-G2 is expressed in the neurons of the developing telencephalon, and the DGS-I gene is involved in the DiGeorge syndrome. The human homolog to rat norbin is likely to be involved in neural plasticity. The LYST:LYST-IP complexes (particularly the LYST:14-3-3. LYST:HS1 protein, LYST:Atrophin-I, LYST:DGS-I, LYST:LIP3, LYST:norbin, LYST-2-:HBF-G2, LYST-2:14-3-3. complexes), derivatives, analogs and fragments thereof, nucleic acids encoding the LYST and LYST-IP genes, anti-LYST:LYST-IP antibodies, and other modulators of LYST:LYST-IP complex activity, can be tested for activity in treating or preventing neurodegenerative disease in *in vitro* and *in vivo* assays.

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In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing neurodegenerative disease by contacting cultured cells that exhibit an indicator of a neurodegenerative disease, such as overexpression of the β-A4 peptide, *in vitro* with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing neurodegenerative disease. Specific examples of cell culture models for neurodegenerative disease include, but are not limited to, cultured rat endothelial cells from affected and nonaffected individuals (Maneiro et al., 1997, Methods Find. Exp. Clin. Pharmacol. 19: 5-12), P19 murine embryonal carcinoma cells (Hung et al., 1992, Proc. Natl. Acad. Sci. USA 89: 9439-9443), and dissociated cell cultures of cholinergic neurons from the nucleus basalis of Meynert (Nakajima et al., 1985, Proc. Natl. Acad. Sci. USA, 82: 6325-6329).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing neurodegenerative disease by administering the Therapeutic to a test animal that exhibits symptoms of a neurodegenerative disease, such as premature development of cognitive deficiencies in transgenic animals expressing  $\beta$ -APP, or that is predisposed to develop symptoms of a neurodegenerative disease; and measuring the change in said symptoms of the neurodegenerative disease after administration of said Therapeutic, wherein a reduction in the severity of the symptoms of the neurodegenerative disease or prevention of the symptoms of the neurodegenerative disease, indicates that the Therapeutic has activity in treating or preventing neurodegenerative disease. Such a test animal can be any one of a number of animal models known in the art for neurodegenerative disease. These models, including those for Alzheimer's Disease and mental retardation of trisomy 21, which accurately mimic the natural human

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neurodegenerative disease (Campbell, et al., 1997, Mol. Psychiatry 2: 125-129; Schultz et al., 1997, Mol. Cell. Biochem. 174: 193-197; Oron et al., 1997, J. Neural. Transm. Suppl. 49: 77-84). Examples of specific models, include but are not limited to the partial trisomy 16 mouse (Holtzman et al., 1996, Proc. Natl. Acad. Sci. USA 93: 13333-13338), bilateral nucleus basalis magnocellularis-lesioned rats (Popovic et al., 1996, Int. J. Neurosci. 86: 281-299), the aged rat (Muir, 1997, Pharmacol. Biochem. Behav. 56: 687-696), the PDAPP transgenic mouse model of Alzheimer's disease (Johnson-Wood et al., 1997, Proc. Natl. Acad. Sci. USA 94: 1550-1555), and experimental autoimmune dementia (Oron et al., 1997, J. Neural Transm. Suppl. 49: 77-84).

#### ASSAYS FOR TREATMENT OF TUMORIGENESIS

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LYST and LYST-2, and several of the identified binding partners of LYST (e.g., 14-3-3 protein, HS-1 protein, estrogen-receptor related protein. Ftel, BMKI, and casein kinase II SU) have roles in the control of cell proliferation and, therefore, cell-transformation and tumorigenesis. Accordingly, methods of the invention are provided for screening LYST:LYST-IP complexes, proteins, and fragments, derivatives and analogs of the foregoing, for activity in altering cell proliferation, cell transformation and/or tumorigenesis in vitro and in vivo.

The LYST:LYST-IP complexes or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins, derivatives, fragments, and analogs thereof, can be assayed for activity to alter (i.e., increase or decrease) cell proliferation in cultured cells in vitro using methods which are well known in the art for measuring cell proliferation. Specific examples of cell culture models include, but are not limited to, lung cancer, primary rat lung tumor cells (Swafford et al., 1997, Mol. Cell. Biol., 17: 1366-1374) and large-cell undifferentiated cancer cell lines (Mabry et al., 1991, Cancer Cells, 3: 53-58), colorectal cell lines for colon cancer (Park and Gazdar, 1996, J. Cell Biochem. Suppl. 24: 131-141), multiple established cell lines for breast cancer (Hambly et al., 1997, Breast Cancer Res. Treat. 43: 247-258; Gierthy et al., 1997, Chemosphere 34: 1495-1505; Prasad and Church, 1997, Biochem. Biophys. Res. Commun. 232: 14-19), a number of well-characterized cell models for prostate cancer (Webber et al., 1996, Prostate, Part 1. 29: 386-394; Part 2, 30: 58-64; and Part 3, 30: 136-142; Boulikas. 1997. Anticancer Res. 17: 1471-1505), for genitourinary cancers, continuous human bladder cancer cell lines (Ribeiro et al., 1997, Int. J. Radiat. Biol. 72: 11-20), organ cultures of transitional cell carcinomas (Booth et al., 1997, Lab Invest. 76: 843-857), and rat progression models (Vet et al., 1997, Biochim. Biophys Acta 1360: 39-44), and established cell lines for leukemias and

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lymphomas (Drexler, 1994, Leuk. Res. 18: 919-927, Tohyama, 1997, Int. J. Hematol. 65: 309-317).

For example, but not by way of limitation, cell proliferation can be assayed by measuring <sup>3</sup>H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes, such as proto-oncogenes (e.g., c-fos and c-myc), by detecting changes in cell cycle markers, etc. Accordingly, one embodiment of the present invention provides a method of screening LYST:LYST-IP complexes, or an individual LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, and fragments, derivatives, and analogs thereof, for activity in altering (i.e., increasing or decreasing) proliferation of cells in vitro, comprising contacting the cells with a LYST:LYST-IP complex, or an individual LYST-2 or novel LIP protein, or a derivative, analog, or fragment thereof, measuring the proliferation of cells that have been so contacted, and comparing said proliferation with the proliferation of cells not so contacted, wherein a change in the level of proliferation in said contacted cells indicates that the complex or protein of the invention has activity to alter cell proliferation.

The LYST:LYST-IP complexes, or LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, derivative, fragment or analog thereof, can also be screened for activity in inducing or inhibiting cell transformation (or progression to malignant phenotype) in vitro. The complexes and proteins of the invention can be screened by contacting either: (i) cells with a normal phenotype (for assaying for cell transformation) or (ii) a transformed cell phenotype (for assaying for inhibition of cell transformation), with the complex or protein of the invention, and examining such cells for acquisition or loss of characteristics associated with a transformed phenotype (i.e., a set of in vitro characteristics associated with a tumorigenic ability in vivo), including, but not limited to, colony formation in soft agar, a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250 kD surface protein, etc. (see Luria et al., 1978, GENERAL VIROLOGY, 3d Ed., John Wiley & Sons, New York, pp. 436-446).

The LYST:LYST-IP complexes, or a LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein, derivative, fragment, or analog thereof, can also be screened for activity to promote or inhibit tumor formation *in vivo* in a non-human test animal. A vast number of animal models of hyperproliferative disorders, including tumorigenesis and metastatic spread, are known in the art (see Table 317-1, Chapter 317, "Principals of Neoplasia," in

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HARRISON'S PRINCIPALS OF INTERNAL MEDICINE, 13th Edition, Isselbacher et al., eds., McGraw-Hill, New York, p. 1814, and Lovejoy et al., 1997, J. Pathol. 181: 130-135). Specific examples include for lung cancer, transplantation of tumor nodules into rats (Wang et al., 1997, Ann. Thorac. Surg. 64: 216-219) or establishment of lung cancer metastases in SCID mice depleted of NK cells (Yono and Sone, 1997, Gan To Kagaku Ryoho 24: 489-494); for colon cancer, colon cancer transplantation of human colon cancer cells into nude mice (Gutman and Fidler, 1995, World J. Surg. 19: 226-234), the cotton top tamarin model of human ulcerative colitis (Warren, 1996, Aliment. Pharmacol. Ther. 10 Supp 12: 45-47) and mouse models with mutations of the adenomatous polyposis tumor suppressor (Polakis, 1997, Biochim. Biophys. Acta 1332: F127-F147); for breast cancer, transgenic models of breast cancer (Dankort and Muller, 1996, Cancer Treat. Res. 83: 71-88; Amundadittir et al., 1996, Breast Cancer Res. Treat. 39: 119-135) and chemical induction of tumors in rats (Russo and Russo, 1996, Breast Cancer Res. Treat. 39: 7-20); for prostate cancer, chemically-induced and transgenic rodent models, and human xenograft models (Royai et al., 1996, Semin. Oncol. 23: 35-40); for genitourinary cancers, induced bladder neoplasm in rats and mice (Oyasu. 1995, Food Chem. Toxicol 33: 747-755) and xenografts of human transitional cell carcinomas into nude rats (Jarrett et al., 1995, J. Endourol. 9: 1-7); and for hematopoietic cancers, transplanted allogeneic marrow in animals (Appelbaum, 1997, Leukemia 11 (Suppl. 4): S15-S17). Further, general animal models applicable to many types of cancer have been described, including, but not restricted to, the p53-deficient mouse model (Donehower, 1996, Semin. Cancer Biol. 7: 269-278), the Min mouse (Shoemaker et al., 1997, Biochem. Biophys. Acta, 1332: F25-F48), and immune responses to tumors in rat (Frey, 1997, Methods, 12: 173-188).

For example, the complexes and proteins of the present invention can be administered to non-human test animals (preferably test animals predisposed to develop a type of tumor) and the non-human test animal subsequently examined for an increased incidence of tumor formation in comparison with controls not administered the complex or protein of the invention.

Alternatively, the complexes and proteins of the present invention can be administered to non-human test animals having tumors (e.g., animals in which tumors have been induced by introduction of malignant, neoplastic, or transformed cells, or by administration of a carcinogen) and subsequently examining the tumors in the test animals for tumor regression in comparison to controls not administered the complex a protein of the present invention.

### 86 ASSAYS FOR TREATMENT OF PIGMENTATION DISORDERS

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LYST protein has been implicated in pigmentation disorders (see *supra*), including oculocutaneous albinism and hypopigmentation. Accordingly, LYST:LYST-IP complexes, and derivatives, analogs, and fragments thereof, nucleic acids encoding the LYST or LYST-2 genes, anti-LYST:LYST-IP antibodies or nucleic acids, and other modulators of the LYST:LYST-IP complex, can be tested for activity in treating or preventing pigmentation disorders in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing pigmentation disorders by contacting cultured cells that exhibit an indicator of a pigmentation reaction, *in vitro* with the Therapeutic, and comparing the level of indicator in the cells so contacted with said level of the indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing pigmentation disorders. Cell models that can be used for such assays include, but are not limited to, *in vitro* studies using cultured vitiligo melanocytes and keratinocytes (Bessou *et al.*, 1997, *Br. J. Dermatol. 137*: 890-897); lines of immortal, severely hypopigmented melanocytes and melanoblasts from mice of the null genotype p(cp)/p(25H) (Sviderskaya *et al.*, 1997, *J. Invest. Dermatol. 108*: 30-34); and organotypic culture of human skin to study melanocyte migration (Le Poole *et al.*, 1994, *Pigment. Cell Res. 7*: 33-43).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing pigmentation disease by administering said Therapeutic to a test animal exhibiting a pigmentation reaction or which test animal does not exhibit a pigmentation reaction and is subsequently challenged with an agent that elicits a pigmentation reaction; and measuring the change in the pigmentation reaction after the administration of said Therapeutic, wherein a reduction in said pigmentation reaction or a prevention of said pigmentation reaction indicates that the Therapeutic has activity in treating or preventing an pigmentation disease.

A number of animal models of pigmentation disorders are known in the art. These models accurately mimic natural human pigmentation diseases. Examples of specific models include, but are not limited to, smyth line (SL) chickens that develop a spontaneous, autoimmune loss of melanocytes (vitiligo) from the feather (Erf et al., 1997, Vet. Immunol. Immunopathol. 58: 335-343); and mouse pale ear (ep) mutation (Gardner et al., Proc. Natl Acad. Sci. U.S.A. 94: 9238-9243).

### ASSAYS FOR TREATMENT OF PLATELET DYSFUNCTION

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LYST protein has been implicated in platelet dysfunction (see *supra*). Accordingly, LYST:LYST-IP complexes, and derivatives, analogs, and fragments thereof, nucleic acids encoding the LYST genes, anti-LYST:LYST-IP antibodies or nucleic acids, and other modulators of the LYST:LYST-IP, can be tested for activity in treating or preventing platelet dysfunction in *in vitro* and *in vivo* assays.

In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing platelet dysfunction by contacting cultured cells *in vitro* that exhibit an indicator of a platelet reaction with the Therapeutic, and comparing the level of indicator in the cells so contacted with said level of the indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing platelet dysfunction.

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing platelet dysfunction by administering said Therapeutic to a test animal exhibiting a platelet reaction or which test animal does not exhibit a platelet reaction and is subsequently challenged with an agent that elicits a platelet reaction; and measuring the change in the platelet reaction after the administration of said Therapeutic, wherein a reduction in said pigmentation reaction or a prevention of said platelet reaction indicates that the Therapeutic has activity in treating or preventing an platelet dysfunction.

A number of animal models of platelet dysfunction are known in the art. These models accurately mimic natural human platelet dysfunction. Examples of specific models include, but are not limited to, a skin bleeding time test in a rat model (MacDonald et al., 1994, Thromb; Res. 76: 535-540), correlation between bleeding time and antithrombotic effect of platelet-suppressive agents in rat experimental model (Suehiro, et al., 1994, Res. Commun. Chem. Pathol. Pharmacol. 83: 157-163), and others.

#### ASSAYS FOR TREATMENT OF VIRAL INFECTIONS

The LYST interactant Importin beta subunit protein is strongly implicated in viral infection mechanisms, including that for the AIDS virus, HIV-1. The nuclear localization signal of the HIV-1 Rev protein mediates specific binding to human Importin-beta. An enormous number of human diseases result from virulent and opportunistic viral infection (see Belshe (Ed.) 1984 Textbook of Human Virology. PSG Publishing, Littleton, MA). Diseases of a wide array of tissues, including the respiratory tract, the CNS, skin, the genitourinary tract, eyes, ears.

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the immune system, the gastrointestinal tract, the musculoskeletal system, affect a vast number of humans of all ages (see Table 328-2 In: Wyngaarden and Smith, 1988, CECIL TEXTBOOK OF MEDICINE, 18th Ed., W.B. Saunders Co., Philadelphia, pp.1750-1753). Accordingly, LYST:LYST-IP complexes, particularly the LYST:Importin beta complex, and derivatives, analogs, and fragments thereof, nucleic acids containing the LYST or LYST-IP gene. in particular the Importin beta gene; anti-LYST:LYST-IP antibodies or nucleic acids, in particular anti-LYST:Importin beta antibodies or nucleic acids, and other modulators of the LYST:LYST-IP complex activity, can be tested for activity in treating or preventing viral diseases in *in vitro* and *in vivo* assays.

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In one embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing viral disease by contacting cultured cells that exhibit an indicator of a viral reaction, e.g., formation of inclusion bodies, in vitro with the Therapeutic, and comparing the level of said indicator in the cells contacted with the Therapeutic with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the Therapeutic has activity in treating or preventing viral disease. Cell models that can be used for such assays include, but are not limited to, viral infection of T lymphocytes (Selin et al., 1996, J. Exp. Med. 183: 2489-2499); hepatitis B infection of dedifferentiated hepatoma cells (Raney et al., 1997, J. Virol. 71: 1058-1071); viral infection of cultured salivary gland epithelial cells (Clark et al., 1994, Autoimmunity 18: 7-14); synchronous HIV-1 infection of CD4+ lymphocytic cell lines (Wainberg et al., 1997, Virology 233: 364-373); viral infection of respiratory epithelial cells (Stark et al., 1996, Human Gene Ther. 7: 1669-1681); and amphotrophic retroviral infection of NIH-3T3 cells (Morgan et al., 1995, J. Virol. 69: 6994-7000).

In another embodiment, a Therapeutic of the invention can be assayed for activity in treating or preventing viral disease by administering said Therapeutic to a test animal having symptoms of a viral infection, such as characteristic respiratory symptoms in animal models of virally-induced asthma, or which test animal does not exhibit a viral reaction and is subsequently challenged with an agent that elicits an viral reaction; and measuring the change in the viral reaction after the administration of said Therapeutic, wherein a reduction in said viral reaction or a prevention of said viral reaction indicates that the Therapeutic has activity in treating or preventing viral disease. Animal models that can be used for such assays include, but are not limited to, respiratory viral infections in guinea pigs (Kudlacz and Knippenberg, 1995, *Inflamm. Res. 44*: 105-110): influenza virus infection of mice (Dobbs *et al.*, 1996, *J. Immunol. 157*: 1870-1877); respiratory syncitial virus infection in lambs (Masot *et al.*, 1996, *Zentralbl. Veterinarmed.* 

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43: 233-243): neurotrophic virus infection of mice (Barna et al., 1996, Virology 223: 331-343); measles infection in hamsters (Fukuda et al., 1994, Acta Otolaryngol. Suppl (Stockh.) 514: 111-116); encephalomyocarditis infection of mice (Hirasawa et al., 1997, J. Virol. 71: 4024-4031); and cytomegalovirus infection of mice (Orange and Biron, 1996, J. Immunol. 156: 1138-1142).

# SCREENING FOR ANTAGONISTS AND AGONISTS OF LYST:LYST-IP COMPLEXES, LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, OR LIP10 PROTEINS

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LYST:LYST-IP complexes, or individual LYST-2 or novel LIP proteins, and derivatives, fragments and analogs thereof, as well as nucleic acids encoding LYST, LYST-2, LYST-IPs, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10, as well as derivatives, fragments and analogs thereof, can be used to screen for compounds that bind to said nucleic acids, proteins, or derivatives, and thus have potential uses as agonists or antagonists of LYST:LYST-IP complexes, or as antagonists or agonists of an individual LYST-2 or novel LIP protein function. The present invention thus provides assays to detect molecules that specifically bind to LYST:LYST-IP complexes, and LYST, LYST-2, LYST-IP, nucleic acids, proteins or derivatives.

For example, recombinant cells expressing both LYST or LYST-2 and/or LYST-IP nucleic acids, or expressing individual LYST-2 or novel LIP nucleic acids, can be used to recombinantly produce the complexes or proteins used in these assays, to screen for molecules that bind or interfere with LYST:LYST-IP complexes, or that interfere with an individual LYST-2 or novel LIP function. In preferred embodiments, peptide analogs that have superior stabilities yet retain the ability to form LYST:LYST-IP complexes, (e.g., LYST or LYST-2 and LYST-IPs modified to be resistant to proteolytic degradation in the binding assay buffers, or resistant to exidative degradation) are used to screen for modulators which are, e.g., molecules generated by substitution of amino acids at proteolytic cleavage sites, such as the use of chemically derivatized amino acids at proteolytic susceptible sites, and replacement of amino acid residues subject to oxidation, i.e., methionine and cysteine.

Molecules (e.g., putative binding partners of a LYST:LYST-IP complex. or of an individual LYST-2 or novel LIP protein) are contacted with the LYST:LYST-IP complex or with an individual LYST-2 or novel LIP protein, or fragment thereof, under conditions conducive to binding, and then molecules that specifically bind to a LYST:LYST-IP complex, or to an

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individual LYST-2 or novel LIP protein, are identified. Similar methods can be used to screen for molecules that bind to LYST or LYST-2 and/or LYST-IP nucleic acids or derivatives.

A particular aspect of the invention relates to identifying molecules that inhibit or promote formation or dissolution or degradation of a LYST:LYST-IP complex, e.g., using the method described for screening inhibitors using the modified yeast two hybrid assay described infra.

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In one embodiment of the present invention, a molecule that modulates activity of LYST or LYST-2 or LYST-1P. or a complex of LYST or LYST-2 and said protein, is identified by contacting one or more candidate molecules with LYST or LYST-2 in the presence of said protein; and measuring the amount of complex that forms between LYST or LYST-2 and said protein; wherein an increase or decrease in the amount of complex that forms relative to the amount that forms in the absence of the candidate molecules indicates that the molecules modulate the activity of LYST or LYST-2 or said protein or said complex of LYST or LYST-2 and said protein. In preferred embodiments, modulators are identified by administering a candidate molecule to a transgenic non-human animal expressing both LYST or LYST-2 and a LYST-IP from promoters that are not the native LYST or LYST-2 or the native LYST-IP promoters, more preferably where the candidate molecule is also recombinantly expressed in the transgenic non-human animal. Alternatively, the method for identifying such modulators can be carried out *in vitro*, preferably with purified LYST or LYST-2, purified LYST-IP, and a purified candidate molecule.

Methods that can be used to carry out the foregoing are commonly known in the art.

Agents to be screened can be provided as mixtures of a limited number of specified compounds, or as compound libraries, peptide libraries and the like. Agents to be screened may also include all forms of antisera, antisense nucleic acids, etc., that can modulate LYST:LYST-IP complex activity, or modulate an individual LYST-2 or novel LIP activity.

By way of example, diversity libraries, such as random or combinatorial peptide or non-peptide libraries can be screened for molecules that specifically bind to a LYST:LYST-IP complex, or to an individual LYST-2 or novel LIP protein. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant libraries (e.g., phage display libraries), and in vitro translation-based libraries.

Examples of chemically synthesized libraries are described in Fodor et al., 1991, Science 251: 767-773; Houghten et al., 1991, Nature 354: 84-86; Lam et al., 1991, Nature 354: 82-84: Medynski, 1994, Bio/Technology 12: 709-710; Gallop et al., 1994, J. Medicinal Chemistry

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37(9): 1233-1251; Ohlmeyer et al., 1993, Proc. Natl. Acad. Sci. USA 90: 10922-10926; Erb et al., 1994, Proc. Natl. Acad. Sci. USA 91: 11422-11426; Houghten et al., 1992, Biotechniques 13: 412: Javawickreme et al., 1994, Proc. Natl. Acad. Sci. USA 91: 1614-1618: Salmon et al., 1993, Proc. Natl. Acad. Sci. USA 90: 11708-11712, and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89: 5381-5383. Examples of phage display libraries are described in Scott and Smith 1990, Science 249: 386-390; Devlin et al., 1990, Science 249: 404-406; Christian, et al., 1992, J. Mol. Biol. 227: 711-718; Lenstra, 1992, J. Immunol. Meth. 152: 149-157; and Kay et al., 1993, Gene 128: 59-65. In vitro translation-based libraries include but are not limited to those described in International Patent Publication WO 91/05058; and Mattheakis et al., 1994, Proc. Natl. Acad. Sci. USA 91: 9022-9026. By way of example of non-peptide libraries, a benzodiazepine library (see, e.g., Bunin et al., 1994, Proc. Natl. Acad. Sci. USA 91: 4708-4712) can be adapted for use. Peptoid libraries (Simon et al., 1992, Proc. Natl. Acad. Sci. USA 89: 9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh et al., 1994, Proc. Natl. Acad. Sci. USA 91: 11138-11142.

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, e.g., the following references, which disclose screening of peptide libraries: Parmley et al., 1989, Adv. Exp. Med. Biol. 251: 215-218; Scott et al., 1990, Science 249: 386-390; Fowlkes et al., 1992, BioTechniques 13: 422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89: 5393-5397; Yu et al., 1994, Cell 76: 933-945; Staudt et al., 1988, Science 241: 577-580; Bock et al., 1992, Nature 355: 564-566; Tuerk et al., 1992, Proc. Natl. Acad. Sci. USA 89: 6988-6992; Ellington et al., 1992, Nature 355: 850-852; U.S. Patent No. 5.096,815; U.S. Patent No. 5.223,409; U.S. Patent No. 5,198,346; and Rebar et al., 1993, Science 263: 671-673.

In a specific embodiment, screening can be carried out by contacting the library members with a LYST:LYST-IP complex, or with a LYST-2. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 protein (or encoding nucleic acid molecule or derivative) immobilized on a solid phase, and harvesting those library members that bind to the protein (or nucleic acid or derivative). Examples of such screening methods, termed "panning" techniques, are described by way of example in Parmley and Smith, 1988. *Gene 73*: 305-318; Fowlkes *et al.*, 1992, *BioTechniques 13*: 422-427; International Patent Publication No. WO 94/18318; and in references cited hereinabove.

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In a specific embodiment, fragments and/or analogs of LYST or LYST-2 or a LYST-IP, especially peptidomimetics, are screened for activity as competitive or non-competitive inhibitors of LYST:LYST-IP complex formation, and thereby inhibit LYST:LYST-IP complex activity. In a preferred embodiment, molecules that bind to a LYST:LYST-IP complex, or to an individual LYST-2 or novel LIP protein, can be screened for by using the modified yeast two hybrid system described *infra*, and exemplified in EXAMPLES, *infra*.

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In one embodiment, agents that modulate (i.e., inhibit, antagonize or agonize)

LYST:LYST-IP complex activity can be screened for using a binding inhibition assay, wherein agents are screened for their ability to inhibit formation of a LYST:LYST-IP complex under aqueous, or physiological, binding conditions in which LYST:LYST-IP complex formation occurs in the absence of the agent to be tested. Agents that interfere with the formation of LYST:LYST-IP complexes are identified as antagonists of complex formation. Agents that eliminate the formation of LYST:LYST-IP complexes are identified as inhibitors of complex formation. Agents that enhance the formation of LYST:LYST-IP complexes are identified as agonists of complex formation.

Methods for screening may involve labeling the complex proteins with radioligands (e.g., 125] or 3H), magnetic ligands (e.g., paramagnetic beads covalently attached to photobiotin acetate), fluorescent ligands (e.g., fluorescein or rhodamine) or enzyme ligands (e.g., luciferase or beta-galactosidase). The reactants that bind in solution can then be isolated by one of many techniques known in the art, including but not restricted to. co-immunoprecipitation of the labeled moiety using antisera against the unlabeled binding partner (or a binding partner labeled with a distinguishable marker from that used on the labeled moiety), immunoaffinity chromatography, size exclusion chromatography, and gradient density centrifugation. In a preferred embodiment, one binding partner is a small fragment or peptidomimetic that is not retained by a commercially available filter. Upon binding, the labeled species is then unable to pass through the filter, providing for a simple assay of complex formation.

Methods commonly known in the art are used to label at least one of the members of the LYST:LYST-IP complex. Suitable labeling includes, but is not limited to, radiolabeling by incorporation of radiolabeled amino acids, e.g., <sup>3</sup>H-leucine or <sup>35</sup>S-methionine, radiolabeling by post-translational iodination with <sup>125</sup>I or <sup>131</sup>I using the chloramine T method. Bolton-Hunter reagents, etc., labeling with <sup>32</sup>P using a kinase and inorganic radiolabeled phosphorous, biotin labeling with photobiotin-acetate and sunlamp exposure, etc. In cases where one of the members of the LYST:LYST-IP complex is immobilized, e.g., as described *infra*, the free species is

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labeled. Where neither of the interacting species is immobilized, each can be labeled with a distinguishable marker such that isolation of both moieties can be followed to provide for more accurate quantitation, and to distinguish the formation of homomeric from heteromeric complexes. Methods that utilize accessory proteins that bind to one of the modified interactants to improve the sensitivity of detection, increase the stability of the complex, etc. are provided.

Typical binding conditions are, for example, but not by way of limitation. in an aqueous salt solution of 10-250 mM NaCl, 5-50 mM Tris-HCl, pH 5-8, and 0.5% Triton X-100 or other detergent that improves the specificity of interaction. Metal chelators and/or divalent cations may be added to improve binding and/or reduce proteolysis. Reaction temperatures may range around 4, 10, 15, 22, 25, 37, or 42 degrees Celsius, and time of incubation is typically at least 15 seconds, but longer times are preferred to allow binding equilibrium to occur. Particular LYST:LYST-IP complexes can be assayed using routine protein binding assays to determine optimal binding conditions for reproducible binding.

The physical parameters of complex formation can be analyzed by quantitation of complex formation using assay methods specific for the label used, e.g., liquid scintillation spectroscopy for radioactivity detection, enzyme activity measurements for enzyme labeling, etc. The reaction results are then analyzed utilizing Scatchard analysis, Hill analysis, and other methods commonly known in the art (see, e.g., PROTEINS, STRUCTURES, AND MOLECULAR PRINCIPLES, 2nd Edition (1993) Creighton, Ed., W.H. Freeman and Company, New York).

In a second common approach to binding assays, one of the binding species is immobilized on a filter, in a microtiter plate well, in a test tube, to a chromatography matrix, etc., either covalently or non-covalently. Proteins can be covalently immobilized using any method well known in the art, for example, but not limited to the method of Kadonaga and Tjian (1986, *Proc. Natl. Acad. Sci. USA 83*: 5889-5893, 1986), *i.e.*, linkage to a cyanogen-bromide derivatized substrate such as CNBr-Sepahrose 4B. Where needed, the use of spacers can reduce steric hindrance by the substrate. Non-covalent attachment of proteins to a substrate include, but are not limited to, attachment of a protein to a charged surface, binding with specific antibodies, binding to a third unrelated interacting protein.

In one embodiment, immobilized LYST or LYST-2 is used to assay for binding with a radioactively-labeled LYST-IP in the presence and absence of a compound to be tested for its ability to modulate LYST:LYST-IP complex formation. The binding partners are allowed to bind under aqueous. or physiological. conditions (e.g., the conditions under which the original interaction was detected). Conversely, in another embodiment, the LYST-IP is immobilized and

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contacted with the labeled LYST or LYST-2 protein or derivative thereof under binding conditions.

Assays of agents (including cell extracts or library pools) for competition for binding of one member of a LYST:LYST-IP complex (or derivatives thereof) with the other member of the LYST:LYST-IP complex (labeled by any means, e.g., those means described supra), are provided to screen for competitors of LYST:LYST-IP complex formation.

In specific embodiments, blocking agents to inhibit non-specific binding of reagents to other protein components, or absorptive losses of reagents to plastics, immobilization matrices, etc., are included in the assay mixture. Blocking agents include, but are not restricted to, bovine serum albumin, beta-casein, nonfat dried milk, Denhardt's reagent, Ficoll, polyvinylpyrolidine, nonionic detergents (NP40, Triton X-100, Tween 20, Tween 80, etc.), ionic detergents (e.g., SDS, LDS, etc.), polyethylene glycol, etc. Appropriate blocking agent concentrations are utilized to allow LYST:LYST-IP complex formation.

After binding is performed, unbound, labeled protein is removed with the supernatant, and the immobilized protein with any bound, labeled protein is washed extensively. The amount of label bound is then quantitated using standard methods known in the art to detect the label.

#### ASSAYS FOR PROTEINS-PROTEIN INTERACTIONS

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One aspect of the present invention provides methods for assaying and screening fragments, derivatives and analogs of LYST or LYST-2 interacting proteins (for binding to a LYST or LYST-2 peptide). Derivatives, analogs and fragments of LYST-IPs that interact with LYST or LYST-2 can be identified by means of a yeast two hybrid assay system (Fields and Song, 1989, *Nature 340*: 245-246 and U.S. Patent No. 5,283,173). Because the interactions are screened for in yeast, the intermolecular protein interactions detected in this system occur under physiological conditions that mimic the conditions in mammalian cells (Chien *et al.*, 1991, *Proc. Natl. Acad. Sci. USA 88*: 9578-9581).

Identification of interacting proteins by the improved yeast two hybrid system is based upon the detection of expression of a reporter gene, the transcription of which is dependent upon the reconstitution of a transcriptional regulator by the interaction of two proteins, each fused to one half of the transcriptional regulator. The "bait" (LYST or LYST-2 or derivative or analog) and "prey" (proteins to be tested for ability to interact with the bait) proteins are expressed as fusion proteins to a DNA binding domain, and to a transcriptional regulatory domain, respectively, or vice versa. In various specific embodiments, the prey has a complexity in the

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range of about 25 to about 100.000. about 100 to about 100.000. about 50,000 to about 100.000. or about 100,000 to about 500.000. For example, the prey population can be one or more nucleic acids encoding mutants of a LYST-IP (e.g., as generated by site-directed mutagenesis or another method of making mutations in a nucleotide sequence). Preferably, the prey populations are proteins encoded by DNA. e.g., cDNA or genomic DNA or synthetically generated DNA. For example, the populations can be expressed from chimeric genes comprising cDNA sequences from an un-characterized sample of a population of cDNA from mammalian RNA.

In a specific embodiment, recombinant biological libraries expressing random peptides can be used as the source of prey nucleic acids.

In another embodiment, the invention provides methods of screening for inhibitors or enhancers of the protein interactants identified herein. Briefly, the protein-protein interaction assay can be carried out as described herein, except that it is done in the presence of one or more candidate molecules. An increase or decrease in reporter gene activity relative to that present when the one or more candidate molecules are absent indicates that the candidate molecule has an effect on the interacting pair. In a preferred method, inhibition of the interaction is selected for (i.e., inhibition of the interaction is necessary for the cells to survive), for example, where the interaction activates the URA3 gene, causing yeast to die in medium containing the chemical 5-fluoroorotic acid (Rothstein. 1983, Meth. Enzymol. 101: 167-180). The identification of inhibitors of such interactions can also be accomplished, for example, but not by way of limitation, using competitive inhibitor assays, as described supra.

In general, proteins of the bait and prey populations are provided as fusion (chimeric) proteins (preferably by recombinant expression of a chimeric coding sequence) comprising each protein contiguous to a pre-selected sequence. For one population, the pre-selected sequence is a DNA binding domain. The DNA binding domain can be any DNA binding domain, as long as it specifically recognizes a DNA sequence within a promoter. For example, the DNA binding domain is of a transcriptional activator or inhibitor. For the other population, the pre-selected sequence is an activator or inhibitor domain of a transcription factor. The regulatory domain alone (not as a fusion to a protein sequence) and the DNA-binding domain alone (not as a fusion to a protein sequence) preferably do not detectably interact (so as to avoid false positives in the assay). The assay system further includes a reporter gene operably linked to a promoter that contains a binding site for the DNA binding domain of the transcriptional activator (or inhibitor). Accordingly, in the present method of the present invention, binding of a LYST or LYST-2 fusion protein to a prey fusion protein leads to reconstitution of a transcriptional activator (or

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inhibitor) which activates (or inhibits) expression of the reporter gene. The activation (or inhibition) of transcription of the reporter gene occurs intracellularly, e.g., in prokaryotic or eukaryotic cells, preferably in cell culture.

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The promoter that is operably linked to the reporter gene nucleotide sequence can be a native or non-native promoter of the nucleotide sequence, and the DNA binding site(s) that are recognized by the DNA binding domain portion of the fusion protein can be native to the promoter (if the promoter normally contains such binding site(s)) or non-native to the promoter. Thus, for example, one or more tandem copies (e.g., 4 or 5 copies) of an appropriate DNA binding site can be introduced upstream of the TATA box in the desired promoter (e.g., in the area of about position -100 to about -400). In a preferred aspect, 4 or 5 tandem copies of the 17 base pair UAS (GAL4 DNA binding site) are introduced upstream of the TATA box in the desired promoter, which is upstream of the desired coding sequence for a selectable or detectable marker. In a preferred embodiment, the GAL1-10 promoter is operably fused to the desired nucleotide sequence; the GAL1-10 promoter already contains 5 binding sites for GAL4.

Alternatively, the transcriptional activation binding site of the desired gene(s) can be deleted and replaced with GAL4 binding sites (Bartel et al., 1993, BioTechniques 14: 920-924, Chasman et al., 1989, Mol. Cell. Biol. 9: 4746-4749). The reporter gene preferably contains the sequence encoding a detectable or selectable marker, the expression of which is regulated by the transcriptional activator, such that the marker is either turned on or off in the cell in response to the presence of a specific interaction. Preferably, the assay is carried out in the absence of background levels of the transcriptional activator (e.g., in a cell that is mutant or otherwise lacking in the transcriptional activator). In one embodiment, more than one reporter gene is used to detect transcriptional activation, e.g., one reporter gene encoding a detectable marker and one or more reporter genes encoding different selectable markers. The detectable marker can be any molecule that can give rise to a detectable signal, e.g., a fluorescent protein or a protein that can be readily visualized or that is recognizable by a specific antibody. The selectable marker can be any protein molecule that confers the ability to grow under conditions that do not support the growth of cells not expressing the selectable marker, e.g., the selectable marker is an enzyme that provides an essential nutrient and the cell in which the interaction assay occurs is deficient in the enzyme and the selection medium lacks such nutrient. The reporter gene can either be under the control of the native promoter that naturally contains a binding site for the DNA binding protein, or under the control of a heterologous or synthetic promoter.

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The activation domain and DNA binding domain used in the assay can be from a wide variety of transcriptional activator proteins, as long as these transcriptional activators have separable binding and transcriptional activation domains. For example, the GAL4 protein of *S. cerevisiae* (Ma et al., 1987, Cell 48: 847-853), the GCN4 protein of *S. cerevisiae* (Hope and Struhl, 1986, Cell 46: 885-894), the ARD1 protein of *S. cerevisiae* (Thukral et al., 1989, Mol. Cell. Biol. 9: 2360-2369), and the human estrogen receptor (Kumar et al., 1987, Cell 51: 941-951), have separable DNA binding and activation domains. The DNA binding domain and activation domain that are employed in the fusion proteins need not be from the same transcriptional activator. In a specific embodiment, a GAL4 or LexA DNA binding domain is employed. In another specific embodiment, a GAL4 or herpes simplex virus VP16 (Triezenberg et al., 1988, Genes Dev. 2: 730-742) activation domain is employed. In a specific embodiment, amino acids 1-147 of GAL4 (Ma et al., 1987, Cell 48: 847-853; Ptashne et al., 1990, Nature 346: 329-331) is the DNA binding domain, and amino acids 411-455 of VP16 (Triezenberg et al., 1988, Genes Dev. 2: 730-742; Cress et al., 1991, Science 251: 87-90) comprise the activation domain.

In a preferred embodiment, the yeast transcription factor GAL4 is reconstituted by protein-protein interaction and the host strain is mutant for GAL4. In another embodiment, the DNA-binding domain is Ace1N and/or the activation domain is Ace1, the DNA binding and activation domains of the Ace1 protein, respectively. Ace1 is a yeast protein that activates transcription from the CUP1 operon in the presence of divalent copper. CUP1 encodes metallothionein, which chelates copper, and the expression of CUP1 protein allows growth in the presence of copper, which is otherwise toxic to the host cells. The reporter gene can also be a CUP1-lacZ fusion that expresses the enzyme beta-galactosidase (detectable by routine chromogenic assay) upon binding of a reconstituted Ace1N transcriptional activator (see Chaudhuri et al., 1995, FEBS Letters 357: 221-226). In another specific embodiment, the DNA binding domain of the human estrogen receptor is used, with a reporter gene driven by one or three estrogen receptor response elements (Le Douarin et al., 1995, Nucl. Acids. Res. 23: 876-878).

The DNA binding domain and the transcriptional activator/inhibitor domain each preferably has a nuclear localization signal (see Ylikomi *et al.*, 1992, *EMBO J. 11*: 3681-3694. Dingwall and Laskey, 1991, *TIBS 16*: 479-481) functional in the cell in which the fusion proteins are to be expressed.

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To facilitate isolation of the encoded proteins, the fusion constructs can further contain sequences encoding affinity tags such as glutathione-S-transferase or maltose-binding protein or an epitope of an available antibody, for affinity purification (e.g., binding to glutathione, maltose, or a particular antibody specific for the epitope, respectively) (Allen et al., 1995, TIBS 20: 511-516). In another embodiment, the fusion constructs further comprise bacterial promoter sequences for recombinant production of the fusion protein in bacterial cells.

The host cell in which the interaction assay occurs can be any cell, prokaryotic or eukaryotic, in which transcription of the reporter gene can occur and be detected, including, but not limited to, mammalian (e.g., monkey, mouse, rat, human, bovine), chicken, bacterial, or insect cells, and is preferably a yeast cell. Expression constructs encoding and capable of expressing the binding domain fusion proteins, the transcriptional activation domain fusion proteins, and the reporter gene product(s) are provided within the host cell, by mating of cells containing the expression constructs, or by cell fusion, transformation, electroporation, microinjection, etc. In a specific embodiment in which the assay is carried out in mammalian cells (e.g., hamster cells), the DNA binding domain is the GAL4 DNA binding domain, the activation domain is the herpes simplex virus VP16 transcriptional activation domain, and the reporter gene contains the desired coding sequence operably linked to a minimal promoter element from the adenovirus E1B gene driven by several GAL4 DNA binding sites (see Fearon et al., 1992, Proc. Natl. Acad. Sci. USA 89: 7958-7962). The host cell used should not express an endogenous transcription factor that binds to the same DNA site as that recognized by the DNA binding domain fusion population. Also, preferably, the host cell is mutant or otherwise lacking in an endogenous, functional form of the reporter gene(s) used in the assay.

Various vectors and host strains for expression of the two fusion protein populations in yeast are known and can be used (see, e.g., U.S. Patent No. 5,1468.614; Bartel et al., 1993, In: CELLULAR INTERACTIONS IN DEVELOPMENT, Hartley, (ed.), Practical Approach Series xviii, IRL Press at Oxford University Press, New York, NY, pp. 153-179; Fields and Sternglanz, 1994, Trends In Genetics 10: 286-292). By way of example but not limitation, yeast strains or derivative strains made therefrom, which can be used are N105, N106, N1051, N1061, and YULH, as described in Examples. infra. Other exemplary strains that can be used in the assay of the invention also include, but are not limited to, the following:

Y190: MATa, ura3-52. his3-200. lys2-801, ade2-101, trpl-901, leu2-3.112, gal4α. gal80α. cyh'2, LYS2::GALl<sub>UAS</sub>-HIS3<sub>TATA</sub>HIS3, URA3::GALl<sub>UAS</sub>-GALl<sub>TATA</sub>-lacZ; see Harper et al.,

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1993, Cell 75: 805-816, available from Clontech. Palo Alto, CA.. Y190 contains HIS3 and lacZ reporter genes driven by GAL4 binding sites.

CG-1945: MATa, ura3-52, his3-200, lys2-801, ade2-101, trpl-901. leu2-3,112, gal4-542, gal80-538, cyh'2. LYS2::GALl<sub>UAS</sub>:HIS3<sub>TATA</sub>HIS3. URA3::GALl<sub>UAS</sub>:-gers(x3)-YC1<sub>TATA</sub>-lacZ, available from Clontech. Palo Alto, CA. CG-1945 contains HIS3 and lacZ reporter genes driven by GAL4 binding sites.

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Y187: MAT-α, ura3-52, his3-200, ade2-101, trp1-901, leu2-3,112, gal4α, gal80α, URA3::GAL1<sub>UAS</sub>-GAL1<sub>TATA</sub>-lacZ, available from Clontech, Palo Alto, CA. Y187 contains a lacZ reporter gene driven by GAL4 binding sites.

SFY526: MATa, ura3-52, his3-200, lys2-801, ade2-101, trp1-901, leu2-3,112, gal4-542, gal80-538, can', URA3::GAL1-lacZ, available from Clontech, Palo Alto, CA. SFY526 contains HIS3 and lacZ reporter genes driven by GAL4 binding sites.

HF7c: MATa, ura3-52, his3-200, lys2-801, ade2-101, trp1-901, leu2-3,112, gal4-542, gal80-538, LYS2::GAL1-HIS3, URA3::GAL1<sub>UAS 1-MERS(x3)</sub>-CYC1-lacZ, available from Clontech, Palo Alto, CA. HF7c contains HIS3 and lacZ reporter genes driven by GAL4 binding sites.

YRG-2: MATa, ura3-52, his3-200, lys2-801, ade2-101, trp1-901, leu2-3,112, gal4-542, gal80-538, LYS2::GAL1<sub>UAS</sub>-GAL1<sub>TATA</sub>-HIS3, URA3::GAL1<sub>UAS</sub>-mers(x3)-CYC1-lacZ, available from Stratagene, La Jolla, CA. YRG-2 contains HIS3 and lacZ reporter genes driven by GAL4 binding sites.

Many other strains commonly known and available in the art can be used.

If not already lacking in endogenous reporter gene activity, cells mutant in the reporter gene may be selected by known methods, or the cells can be made mutant in the target reporter gene by known gene-disruption methods prior to introducing the reporter gene (Rothstein, 1983, Meth. Enzymol. 101: 202-211).

In a specific embodiment, plasmids encoding the different fusion protein populations can be introduced simultaneously into a single host cell (e.g., a haploid yeast cell) containing one or more reporter genes, by co-transformation, to conduct the assay for protein-protein interactions. Or, preferably, the two fusion protein populations are introduced into a single cell either by mating (e.g., for yeast cells) or cell fusions (e.g., of mammalian cells). In a mating type assay, conjugation of haploid yeast cells of opposite mating type that have been transformed with a binding domain fusion expression construct (preferably a plasmid) and an activation (or inhibitor) domain fusion expression construct (preferably a plasmid), respectively, will deliver both constructs into the same diploid cell. The mating type of a yeast strain may be manipulated

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by transformation with the HO gene (Herskowitz and Jensen. 1991. Meth. Enzymol. 194: 132-146).

In a preferred embodiment, a yeast interaction mating assay is employed using two different types of host cells. strain-type a and alpha of the yeast *Saccharomyces cerevisiae*. The host cell preferably contains at least two reporter genes, each with one or more binding sites for the DNA-binding domain (e.g., of a transcriptional activator). The activator domain and DNA binding domain are each parts of chimeric proteins formed from the two respective populations of proteins. One strain of host cells, for example the a strain, contains fusions of the library of nucleotide sequences with the DNA-binding domain of a transcriptional activator, such as GAL4. The hybrid proteins expressed in this set of host cells are capable of recognizing the DNA-binding site in the promoter or enhancer region in the reporter gene construct. The second set of yeast host cells, for example, the alpha strain, contains nucleotide sequences encoding fusions of a library of DNA sequences fused to the activation domain of a transcriptional activator.

In a preferred embodiment, the fusion protein constructs are introduced into the host cell as a set of plasmids. These plasmids are preferably capable of autonomous replication in a host yeast cell and preferably can also be propagated in *E. coli*. The plasmid contains a promoter directing the transcription of the DNA binding or activation domain fusion genes, and a transcriptional termination signal. The plasmid also preferably contains a selectable marker gene, permitting selection of cells containing the plasmid. The plasmid can be single-copy or multi-copy. Single-copy yeast plasmids that have the yeast centromere may also be used to express the activation and DNA binding domain fusions (Elledge *et al.*, 1988, *Gene 70*: 303-312).

In another embodiment, the fusion constructs are introduced directly into the yeast chromosome via homologous recombination. The homologous recombination for these purposes is mediated through yeast sequences that are not essential for vegetative growth of yeast, e.g., the MER2, MER1, ZIP1, REC102, or ME14 gene.

Bacteriophage vectors can also be used to express the DNA binding domain and/or activation domain fusion proteins. Libraries can generally be prepared faster and more easily from bacteriophage vectors than from plasmid vectors.

In a specific embodiment, the present invention provides a method of detecting one or more protein-protein interactions comprising (a) recombinantly expressing LYST or LYST-2 or a derivative or analog thereof in a first population of yeast cells being of a first mating type and comprising a first fusion protein containing the LYST or LYST-2 sequence and a DNA binding

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domain, wherein said first population of yeast cells contains a first nucleotide sequence operably linked to a promoter driven by one or more DNA binding sites recognized by said DNA binding domain such that an interaction of said first fusion protein with a second fusion protein, said second fusion protein comprising a transcriptional activation domain, results in increased transcription of said first nucleotide sequence; (b) negatively selecting to eliminate those yeast cells in said first population in which said increased transcription of said first nucleotide sequence occurs in the absence of said second fusion protein; (c) recombinantly expressing in a second population of yeast cells of a second mating type different from said first mating type, a plurality of said second fusion proteins, each second fusion protein comprising a sequence of a fragment, derivative or analog of a LYST-IP and an activation domain of a transcriptional activator, in which the activation domain is the same in each said second fusion protein; (d) mating said first population of yeast cells with said second population of yeast cells to form a third population of diploid yeast cells, wherein said third population of diploid yeast cells contains a second nucleotide sequence operably linked to a promoter driven by a DNA binding site recognized by said DNA binding domain such that an interaction of a first fusion protein with a second fusion protein results in increased transcription of said second nucleotide sequence, in which the first and second nucleotide sequences can be the same or different; and (e) detecting said increased transcription of said first and/or second nucleotide sequence, thereby detecting an interaction between a first fusion protein and a second fusion protein.

In a preferred embodiment, the bait LYST or LYST-2 sequence and the prey library of chimeric genes are combined by mating the two yeast strains on solid media for a period of approximately 6-8 hours. In a less preferred embodiment, the mating is performed in liquid media. The resulting diploids contain both kinds of chimeric genes, *i.e.*, the DNA-binding domain fusion and the activation domain fusion.

Preferred reporter genes include the *URA3*, *HIS3* and/or the *lacZ* genes (see. *e.g.*, Rose and Botstein. 1983, *Meth. Enzymol. 101*: 167-180) operably linked to GAL4 DNA-binding domain recognition elements. Other reporter genes comprise the functional coding sequences for, but not limited to, Green Fluorescent Protein (GFP) (Cubitt *et al.*, 1995. *Trends Biochem. Sci. 20*: 448-455). luciferase, *LEU2*, *LYS2*. *ADE2*. *TRP1*, *CAN1*. *CYH2*, *GUS*. *CUP1* or chloramphenicol acetyl transferase (*CAT*). Expression of LEU2, LYS2, ADE2 and TRP1 are detected by growth in a specific defined media: GUS and CAT can be monitored by well known enzyme assays: and CAN1 and CYH2 are detected by selection in the presence of canavanine and cycloheximide. With respect to GFP, the natural fluorescence of the protein is detected.

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In a specific embodiment, transcription of the reporter gene is detected by a linked replication assay. For example, as described by Vasavada et al., 1991, Proc. Natl. Acad. Sci. USA 88: 10686-10690, expression of SV40 large T antigen is under the control of the E1B promoter responsive to GAL4 binding sites. The replication of a plasmid containing the SV40 origin of replication, indicates the reconstruction of the GAL4 protein and a protein-protein interaction. Alternatively, a polyoma virus replicon can be employed (Vasavada et al., 1991, Proc. Natl. Acad. Sci. USA 88: 10686-10690).

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In another embodiment, the expression of reporter genes that encode proteins can be detected by immunoassay, *i.e.*, by detecting the immunospecific binding of an antibody to such protein, which antibody can be labeled, or alternatively, which antibody can be incubated with a labeled binding partner to the antibody, so as to yield a detectable signal. Alam and Cook (1990, *Anal. Biochem. 188*: 245-254) disclose non-limiting examples of detectable marker genes that can be operably linked to a transcriptional regulatory region responsive to a reconstituted transcriptional activator, and thus used as reporter genes.

The activation of reporter genes like URA3 or HIS3 enables the cells to grow in the absence of uracil or histidine, respectively, and hence serves as a selectable marker. Thus, after mating, the cells exhibiting protein-protein interactions are selected by the ability to grow in media lacking a nutritional component, such as uracil or histidine (referred to as -URA (minus URA) and -HIS (minus HIS) medium, respectively). The -HIS medium preferably contains 3amino-1,2,4-triazole (3-AT), which is a competitive inhibitor of the HIS3 gene product, and thus, requires higher levels of transcription in the selection (see. Durfee et al., 1993, Genes Dev. 7: 555-569). Similarly, 6-azauracil, which is an inhibitor of the URA3 gene product, can be included in -URA medium (Le Douarin et al., 1995, Nucl. Acids Res. 23: 876-878). URA3 gene activity can also be detected and/or measured by determining the activity of its gene product, orotidine-51-monophosphate decarboxylase (Pierrat et al., 1992, Gene 119: 237-245, Wolcott et al., 1966, Biochem. Biophys. Acta 122: 532-534). In other embodiments of the present invention. the activities of the reporter genes like GFP or lacZ are monitored by measuring a detectable signal (e.g., fluorescent or chromogenic, respectively) that results from the activation of these reporter genes. For example, lacZ transcription can be monitored by incubation in the presence of a chromogenic substrate, such as X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside), of its encoded enzyme,  $\beta$ -galactosidase. The pool of all interacting proteins isolated by this manner from mating the LYST or LYST-2 sequence product and the library identifies the "LYST interactive population".

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In a preferred embodiment of the present invention, false positives arising from transcriptional activation by the DNA binding domain fusion proteins in the absence of a transcriptional activator domain fusion protein are prevented or reduced by negative selection for such activation within a host cell containing the DNA binding fusion population, prior to exposure to the activation domain fusion population. By way of example, if such cell contains URA3 as a reporter gene, negative selection is carried out by incubating the cell in the presence of 5-fluoroorotic acid (5-FOA, which kills URA+ cells (Rothstein, 1983, Meth. Enzymol. 101: 167-180). Hence, if the DNA-binding domain fusions by themselves activate transcription, the metabolism of 5-FOA will lead to cell death and the removal of self-activating DNA-binding domain hybrids.

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Negative selection involving the use of a selectable marker as a reporter gene and the presence in the cell medium of an agent toxic or growth inhibitory to the host cells in the absence of reporter gene transcription is preferred, since it allows a higher rate of processing than other methods. As will be apparent, negative selection can also be carried out on the activation domain fusion population prior to interaction with the DNA binding domain fusion population, by similar methods, either alone or in addition to negative selection of the DNA binding fusion population.

Negative selection can also be carried out on the recovered LYST:LYST-IP complex by known methods (see, e.g., Bartel et al., 1993, BioTechniques 14: 920-924) although pre-negative selection (prior to the interaction assay), as described above, is preferred. For example, each plasmid encoding a protein (peptide or polypeptide) fused to the activation domain (one-half of a detected interacting complex) can be transformed back into the original screening strain, either alone or with a plasmid encoding only the DNA-binding domain, the DNA-binding domain fused to the detected interacting protein, or the DNA-binding domain fused to a protein that does not affect transcription or participate in the protein-protein interaction. A positive interaction detected with any plasmid other than that encoding the DNA-binding domain fusion to the detected interacting protein is deemed a false positive and is eliminated from the screen.

In a preferred embodiment, the LYST or LYST-2 plasmid population is transformed in a yeast strain of a first mating type (a or alpha), and the second plasmid population (containing the library of DNA sequences) is transformed in a yeast strain of a different mating type. Both strains are preferably mutant for *URA3* and *HIS3*, and contain *HIS3*, and optionally *lacZ*, as reporter genes. The first set of yeast cells are positively selected for the LYST plasmids and are negatively selected for false positives by incubation in medium lacking the selectable marker

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(e.g., tryptophan) and containing 5-FOA. Yeast cells of the second mating type are transformed with the second plasmid population, and are positively selected for the presence of the plasmids containing the library of fusion proteins. Selected cells are pooled. Both groups of pooled cells are mixed together and mating is allowed to occur on a solid phase. The resulting diploid cells are then transferred to selective media that selects for the presence of each plasmid and for activation of reporter genes.

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In a preferred embodiment of the invention, after an interactive population is obtained, the DNA sequences encoding the pairs of interactive proteins are isolated by a method wherein either the DNA-binding domain hybrids or the activation domain hybrids are amplified, in separate respective reactions. Preferably, the amplification is carried out by polymerase chain reaction (PCR) (see, U.S. Patent Nos. 4,683,202; 4,683,195; and 4,889,818; Innis *et al.*, 1990, PCR PROTOCOLS. Academic Press. Inc., San Diego, CA) using pairs of oligonucleotide primers specific for either the DNA-binding domain hybrids or the activation domain hybrids. This PCR reaction can also be performed on pooled cells expressing interacting protein complexes, preferably pooled arrays of interactants. Other amplification methods known in the art can be used, including but not limited to ligase chain reaction (see European Patent No. 320,308), use of Qß replicase, or methods listed in Kricka *et al.*, 1995, MOLECULAR PROBING, BLOTTING, AND SEQUENCING, Academic Press, New York, Chapter 1.

The plasmids encoding the DNA-binding domain hybrid and the activation domain hybrid proteins can also be isolated and cloned by any of the methods well known in the art. For example, but not by way of limitation, if a shuttle (e.g., yeast to E. coli) vector is used to express the fusion proteins, the genes can be recovered by transforming the yeast DNA into E. coli and recovering the plasmids from E. coli (see, e.g., Hoffman et al., 1987, Gene 57: 267-272). Alternatively, the yeast vector can be isolated, and the insert encoding the fusion protein subcloned into a bacterial expression vector, for growth of the plasmid in E. coli.

## PHARMACEUTICAL COMPOSITIONS AND THERAPEUTIC/PROPHYLACTIC ADMINISTRATION

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human. In a specific embodiment, a non-human mammal is the subject.

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Formulations and methods of administration that can be employed when the Therapeutic comprises a nucleic acid are described above; additional appropriate formulations and routes of administration can be selected from among those described hereinbelow.

Various delivery systems are known and can be used to administer a Therapeutic of the invention. e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the Therapeutic, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262: 4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (see Langer, 1990, *Science 249*: 1527-1533, Treat *et al.*, 1989, In: LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.). Liss, New York, pp. 353-365, Lopez-Berestein, ibid., pp. 317-327, see generally ibid.).

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra. Sefton, 1987, CRC Crit. Ref. Biomed. Eng. 14: 201, Buchwald et al., 1980, Surgery 88: 507. Saudek et al., 1989, N. Engl.

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J. Med. 321: 574). In another embodiment, polymeric materials can be used (see Langer and Wise (eds.), 1979. "MEDICAL APPLICATIONS OF CONTROLLED RELEASE", CRC Pres., Boca Raton, Florida: Smolen and Ball (eds.), 1984, CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Wiley, New York: Ranger and Peppas, 1983, J. Macromol. Sci. Rev. Macromol. Chem. 23: 61. See also Levy et al., Science 228: 190, During et al., 1989, Ann. Neurol. 25: 351. Howard et al., 1989, J. Neurosurg. 71: 105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, 1984, In Medical Applications of Controlled Release, supra. Vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, Science 249: 1527-1533.

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In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun, Biolistic, Dupont), or by coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, *e.g.*, Joliot *et al.*, 1991, *Proc. Natl. Acad. Sci. USA 88*: 1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression. by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk,

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glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsions, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulations can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in REMINGTON'S PHARMACEUTICAL SCIENCES, by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water or saline for injection can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the present invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays optionally may be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness

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of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to about 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of about 0.5% to about 10% by weight; oral formulations preferably contain about 10% to about 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

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#### ANIMAL MODELS

The present invention also provides animal models. In one embodiment, animal models for diseases and disorders involving LYST:LYST-IP complexes, and individual LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 proteins are provided. These include, but are not limited to, atopic disorders including asthma, autoimmune disorders, degenerative disorders including neurodegenerative disorders, cell proliferative disorders including tumorigenesis and tumor spread, disorders caused by viral infection, disorders involving pigmentation disorders, and disorders caused by platelet dysfunction. Such animals can be initially produced by promoting homologous recombination or insertional mutagenesis between LYST or LYST-2 and/or LYST-IP genes, or between an individual LYST-2 or novel LIP gene in the chromosome, and exogenous LYST or LYST-2 and/or LYST-IP genes, or individual LYST-2 or novel LIP genes that have been rendered biologically inactive or deleted (preferably by insertion of a heterologous sequence, e.g., an antibiotic resistance gene). In a preferred aspect, homologous recombination is carried out by transforming embryo-derived stem (ES) cells with a vector containing the insertionally inactivated LYST or LYST-2 and/or LYST-IP gene, or an individual LYST-2 or novel LIP gene, such that homologous recombination occurs. followed by injecting the ES cells into a blastocyst, and implanting the blastocyst into a foster mother, followed by the birth of the chimeric animal ("knockout animal") in which a LYST or LYST-2

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and/or a LYST-IP gene. or an individual LYST-2 or novel LIP gene, has been inactivated or deleted (see Capecchi, 1989, Science 244: 1288-1292). The chimeric animal can be bred to produce additional knockout animals. Such animals can be mice, hamsters, sheep, pigs, cattle, etc., and are preferably non-human mammals. In a specific embodiment, a knockout mouse is produced.

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Such knockout animals are expected to develop or be predisposed to developing diseases or disorders involving, but not restricted to, cell proliferative disorders including cancer and benign hypertrophy, various disorders involving cellular apoptosis and cellular differentiation, autoimmune diseases, etc., and thus can have use as animal models of such diseases, viral diseases and disorders, e.g., to screen for or test molecules (e.g., potential Therapeutics) for the ability to inhibit cell proliferative, autoimmune, viral and other diseases.

In a different embodiment of the invention, transgenic animals that have incorporated and express (or overexpress or misexpress) a functional LYST or LYST-2 and/or LYST-IP gene, e.g., by introducing the LYST or LYST-2 and/or LYST-IP genes under the control of heterologous promoters (i.e., promoters that are not the native LYST or LYST-2 or LYST-IP promoter) that either overexpress the protein or proteins, or express them in tissues not normally expressing the complexes or proteins, can have use as animal models of diseases and disorders characterized by clevated levels of a LYST:LYST-IP complex, or by elevated levels of an individual LYST-2 or novel LIP protein. Such animals can be used to screen for or test molecules for the ability to treat or prevent the diseases and disorders cited above.

In one embodiment, the invention provides a recombinant non-human animal in which both the endogenous LYST or LYST-2 gene and an endogenous LYST-IP gene have been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof. In another embodiment, the invention provides a recombinant non-human animal containing both a LYST gene or LYST-2 gene and a LYST-IP gene, in which the LYST or LYST-2 gene is under the control of a promoter that is not the native LYST or LYST-2 gene promoter and the LYST-IP gene is under the control of a promoter that is not the native LYST-IP gene promoter. In a specific embodiment, the invention provides a recombinant non-human animal containing a transgene comprising a nucleic acid sequence encoding a chimeric protein comprising a fragment of LYST or LYST-2 of at least 6 amino acids fused via a covalent bond to a fragment of a LYST-IP of at least 6 amino acids.

The invention also provides a recombinant non-human animal in which an endogenous LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, or LIP10 gene has been deleted

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or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof.

#### SPECIFIC EXAMPLES

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# **EXAMPLE 1: IDENTIFICATION OF LYST:LYST-IP COMPLEXES**

A modified, improved yeast two hybrid system was used to identify protein interactions. Yeast is an eukaryote, and therefore, any intramolecular protein interactions detected in this type of system demonstrate protein interactions that occur under physiological conditions (Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88: 9578-9581). Expression vectors were constructed to encode two hybrid proteins. For a "forward" screen, one hybrid consisted of the DNA binding domain of the yeast transcriptional activator Gal4 fused to a portion of LYST or LYST-2. The other hybrid consisted of the Gal4 activator domain fused to "prey" protein sequences encoded by a mammalian cDNA library. In a "reverse" screen, the portion of LYST was fused to the Gal4 activator domain, and the prey protein sequences of the mammalian cDNA library were fused to the DNA binding domain, but the assay was otherwise identically performed. Each of the vectors was then inserted into complementary (a and alpha) mating types of yeast using methods known in the art (Chien et al., 1991, Proc. Natl. Acad. Sci. USA 88: 9578-9581). Mating was carried out to express both vector constructs within the same yeast cells, thus allowing interaction to occur. Interaction between the bait and prey domains led to transcriptional activation of reporter genes containing cis-binding elements for Gal4. The reporter genes encoding the indicator protein beta-galactosidase, and metabolic markers for uracil and histidine auxotrophy, were included in specific fashion in one or the other of the yeast strains used in the mating. In this way, yeast were selected for successful mating, expression of both fusion constructs, and expression of LYST, LYST-2, or LYST-IPs. Yeast clones that contained interacting proteins were picked and grown in individual wells of microtiter plates. The plasmids containing the LYST-IP sequences were then isolated and characterized.

#### Human cDNA libraries used in the yeast two hybrids system screens.

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The prey cDNAs were obtained from a fetal brain cDNA library of 1 x 10<sup>7</sup> independent isolates (Clontech #HL4029AH. Palo Alto, CA). The library was synthesized from Xho1-dT15 primed fetal brain mRNA (from five male/female 19-22 week fetuses) that was directionally cloned into either pAD-GAL4, a yeast Gal4 activation domain cloning vector including the

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LEU2 gene for selection in yeast deficient in leucine biosynthesis, or pBD-GAL4, a yeast Gal4 DNA-binding domain cloning vector including the TRP1 gene for selection in yeast deficient in tryptophane biosynthesis.

Human adult heart, adult keratinocyte, fetal liver and fetal kidney plasmid cDNA libraries in the pAD-GAL4 vector pACT2 (Clontech #HL4042AH, HL4030AH, HL4029AH, HL4008AB) were amplified and plasmids purified. N106r yeast cells were transformed with 200 µg of the pasmid DNA and 10<sup>7</sup> yeast transformants were used in screens.

#### Forward and reverse screens.

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Four forward screens were used to test the interaction of prey cDNA products against an array of bait proteins, one of which was encoded by the LYST nucleotide sequence of nucleotides 190-1056 (GenBank Accession No. U67615) (see also Table I). The bait fragment 190-1056 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC ATG AGC ACC GAC AGT AAC TCA C-3' (SEQ ID NO:25) and the reverse primer 5'-G GAA TTC TCA TAG TGT GGG CAC TAC ACT GG-3' (SEQ ID NO:26) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGBT9BS (Yang et al., 1995, Nucleic Acids Res. 23: 1152-1156). A second bait was encoded by the LYST nucleotide sequence of nucleotides 3190-4032 (GenBank Accession No. U67615) and was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC CTG TTC AGA AGT CAC AAA GAG G-3' (SEQ ID NO:27) and the reverse primer 5'-G GAA TTC TCA TTG AGT GAG GTT TTC GAG TAA G-3' (SEQ ID NO:28) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGBT9BS (Yang et al., 1995). A third bait was encoded by the LYST nucleotide sequence of nucleotides 6586-7449 (GenBank Accession No. U67615) and was amplified from the full length LYST cDNA by PCR using the forward 5'-GTG GAT CCC CCA TAT GAA GGA GAG AAC TCC TCT AAT ATT ATT CCA-3' (SEQ ID NO:29) and the reverse primer 5'-G GAA TTC TCA TCC CAT GTT TCT CAC ATC TTC CAG-3' (SEQ ID NO:30) by standard techniques. A fourth bait was encoded by the LYST nucleotide sequence of nucleotides 9037-9585 (GenBank Accession No. U67615) and was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC GAT CCA ACA GAA GGG CCA AAT C-3' (SEQ ID NO:31) and the reverse primer 5'-G GAA TTC TCA TGT CAG AGC GGT GAT GTT ACC-3' (SEQ ID NO:32) by standard techniques. The amplified fragment was cloned between the BamHl and EcoRl restriction sites of pGBT9BS (Yang et al., 1995).

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Seven reverse screens were used to test the interaction of prey cDNA products against an array of bait proteins. The baits were encoded by the LYST nucleotide sequence (GenBank Accession No. U67615) of nucleotides 190-1056, 774-1424, 4009-4821, 6586-7449, 9037-9585, 9502-10590 (BEACH domain), 10576-11611 (WD-40 domain), encoding different portions of the LYST protein (GenBank Accession No. U67615). The bait fragment 190-1056 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC ATG AGC ACC GAC AGT AAC TCA C-3' (SEQ ID NO:25) and the reverse primer 5'-G GAA TTC TCA TAG TGT GGG CAC TAC ACT GG-3' (SEQ ID NO:26) by standard techniques. The amplified fragment was cloned between the BamHI and Eco RI restriction sites of pGAD-GH (Clonentech): The bait fragment 774-1424 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC ACA CTA ACT GAG TTC CTA GCA GGC TTT GGG GAC TGC-3' (SEQ ID NO:33) and the reverse primer 5'-GAG AGA GAG AGA GAG AGA CTC GAG TCA ATC CAT ACA ACA GCA TAT TCC AAT G-3' (SEQ ID NO:34) by standard techniques. The amplified fragment was cloned between the BamHI and Xhol restriction sites of pGAD-GH (Clonentech). The bait fragment 4009-4821 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC CTC GAA AAC CTC ACT CAA GGG-3' (SEQ ID NO:35) and the reverse primer 5'-G GAA TTC TCA GGA GCC CAG TGA AAT TAT ATG-3' (SEQ ID NO:36) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGAD-GH (Clonentech). The bait fragment 6586-7449 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC CCA TAT GAA GGA GAG AAC TCC TCT AAT ATT ATT CCA-3' (SEQ ID NO:29) and the reverse primer 5'-G GAA TTC TCA TCC CAT GTT TCT CAC ATC TTC CAG-3' (SEQ ID NO:30) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGAD-GH (Clonentech, Palo Alto, CA). The bait fragment 9037-9585 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC GAT CCA ACA GAA GGG CCA AAT C-3' (SEQ ID NO:31) and the reverse primer 5'-G GAA TTC TCA TGT CAG AGC GGT GAT GTT ACC-3' (SEQ ID NO:32) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGAD-GH (Clonentech). The bait fragment 9502-10590 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC AAG GTT CGT GAT GAT GTA TAC CAC-3' (SEQ ID NO:37) and the reverse primer 5'-G GAA TTC TCA CAA GCC TTT TAT CCA TGA CAA AGG-3' (SEQ ID NO:38) by standard techniques. The amplified fragment was cloned between the BamHl and

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EcoRI restriction sites of pGAD-GH. The bait fragment 10576-11611 was amplified from the full length LYST cDNA by PCR using the forward primer 5'-GTG GAT CCC TGG ATA AAA GGC TTG AAA TGG GG-3' (SEQ ID NO:39) and the reverse primer 5'-G GAA TTC TCA T GAA GTT CAT TCG CAT TCA CCC-3' (SEQ ID NO:40) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGAD-GH.

One reverse screen was used to test the interaction of prey cDNA products against a LYST-2 bait protein (FIG. 1, SEQ ID NO:2). The baits were encoded by the LYST-2 nucleotide sequence (FIG. 1, SEQ ID NO:1) of nucleotides 3-794. The bait fragment 3-794 was amplified from the full length LYST-2 cDNA by PCR using the forward primer 5'-GCG GAA CTA GTG ACT TCT GAT GTA AAG GAA C-3' (SEQ ID NO:41) and the reverse primer 5'-GCG GAA TTC TCA ATA GCG ATT ATC TGC GTG TAC-3' (SEQ ID NO:42) by standard techniques. The bait fragment 774-1424 was amplified from the full length LYST-2 cDNA by PCR using the forward primer 5'-GCG GAA CTA GTG GTA ACA GCA GAT AAT CGC TAT-3' (SEQ ID NO:43) and the reverse primer 5'-GCG GAA TTC TCA CCA GGG TTA AAT GTA GAG TTG-3' (SEQ ID NO:44) by standard techniques. The amplified fragment was cloned between the BamHI and EcoRI restriction sites of pGAD-WM. The bait sequence was confirmed by nucleic acid sequencing to confirm that PCR amplification reproduced an accurate copy of the *LYST* or *LYST-2* sequence. This test determined that, as predicted, the bait sequence encoded an interacting domain identical to human LYST or LYST-2.

In the forward screens the nucleic acid encoding the introduced bait was expressed by lithium acetate/polyethylene glycol transformation (Ito et al., 1983, J. Bacteriol, 153: 163-168) into the yeast strain YULH (mating type a, ura3, his3, lys2, Ade2, trp1, leu2, gal4, gal80, GAL1-URA3, GAL1-lacZ), while the prey sequences were introduced by transformation into the yeast strain N106r (mating type alpha, ura3, his3, ade2, trp1, leu2, gal4, gal80, cyh', Lys2::GAL1<sub>UAS</sub>-HIS3<sub>TATA</sub>-HIS3, ura3::GAL1<sub>UAS</sub>-GAL<sub>TATA</sub>-lacZ). For the reverse screens, LYST baits in the pAD-Gal4 plasmid were transformed into N106r, while the prey cDNA library fused to the pBD-Gal4 plasmid was transformed in YULH. The two transformed populations were then mated using standard methods in the art (Sherman et al., eds., 1991, GETTING STARTED WITH YEAST, Vol. 194, Academic Press, New York). Briefly, cells were grown until mid-to-late log phase on media that selected for the presence of the appropriate plasmids. The two mating strains, alpha and a, were then diluted in YAPD media (Sherman et al., eds., 1991, GETTING STARTED WITH YEAST, Vol. 194, Academic Press, New York), filtered onto nitrocellulose membranes, and incubated at 30°C for 6-8 hours. The cells were then transferred to media selective for the

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desired diploids. *i.e.*. yeast harboring reporter genes for beta-galactosidase, uracil auxotrophy, and histidine auxotrophy, and expression of the vectors encoding the bait and prey. The mating products were plated on SC (synthetic complete) media (Kaiser, Michaelis and Mitchell, Eds. 1994, METHODS IN YEAST GENETICS, 1994 Ed., Cold Spring Harbor Laboratory Press, New York, p.209) lacking adenine and lysine (to select for successful mating), leucine and tryptophan (to select for expression of genes encoded by both the bait and prey plasmids), and uracil and histidine (to select for protein interactions). This medium is herein referred to as SCS medium, for SC Selective medium.

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Selected clones were tested for expression of \(\beta\)-galactosidase to confirm the formation of a LYST:LYST-IP interaction. Filter-lift \(\beta\)-galactosidase assays were performed as modified from the protocol of Breeden and Nasmyth, 1985, Cold Spring Harbor Quant. Biol. 50: 643-650. Colonies were patched onto SCS plates, grown overnight, and replica plated onto Whatman No. 1 filters. The filters were then assayed for \(\beta\)-galactosidase activity. Colonies that were positive turned a visible blue and thus represented LYST:target protein interactions.

Cells in colonies positive for protein interaction contained a mixture of DNA-binding and activation-domain plasmids. These cells were regrown as single isolates in individual wells of 96-well plates. Ten microliters of each isolate was lysed, the inserts within the pAD-GAL4 or pGAD-GH for the activation domain plasmids and pBD-GAL4 or pGBT9BS plasmids were amplified by polymerase chain reaction using primers specific for the flanking sequences of each vector, and approximately 300 amino-terminal bases of each insert was determined using an ABI 377 sequenator.

Comparison to known sequences was made using the "BLAST" software program publicly available through the National Center for Biotechnology Information. A summary of all LYST and LYST-2 interacting proteins including the corresponding interacting domain of LYST, the name of the interacting protein, the library used, the start sites of the interaction and the number of hits per isolate is shown in Table I. The determined nucleic acid sequences and corresponding amino acid sequences of LYSY-2 and novel LIP mentioned interacting proteins are shown in FIGS. 1-11, respectively.

# **TABLE I**

Table I a. Protein interactions identified by forward yeast two-hybrid screens using LYST baits

BINDING	LYST-IP NAME	LYST-IP	HUMAN	START OF	# OF HITS
DOMAIN	(GenBank #)	FUNCTION	ACTIVATION	ISOLATES.	PER
FRAGMENT.			DOMAIN	bp*	ISOLATE
bp*			LIBRARY		
LYST	Casein kinase II B	Phosphorylation:	Fetal liver		2
	-subunit	vesicular			_
190-1056	(M30448)	trafficking and			
		signal transduction			
	14-3-3 protein	Vesicular.	Feal liver	185	1
1	(X56468)	trafficking and	Adult heart	221	1
		signal transduction	Fetal brain	290	1
			Fetal liver	299	2
			Fetal brain	308	l
		÷	Fetal liver	322	1
			Fetal brain	342	l
LYST	OS-9 Precursor	Amplified in	Keratinocyte	1607	3
3190-4032 ·	(U41635)	sarcomas	·		
	Casein kinase II β	Phosphorylation:	Fetal brain	1	28
	- subunit	vesicular	Fetal liver	1	12
	(M30448)	trafficking and	Adult	ì	11
		signal transduction	keratinocyte		
			Adult heart	1	14
			Fetal kidney	2	1
			Adult keratinocyte	3	1
			Fetal brain	5	1
			Fetal brain	35	1
			Fetal brain	86	3
	Troponin I	Muscle filament	Adult heart	ı	6
	(X54163)	contraction	Adult heart	75	3
LYST	Fie-1 (M847)1)	Ribosomal protein	Fetal Brain	51	2

PCT/US99/06831

4819-5700		S3a: fos			
		transformation		1	
	1	effector gene.			
LYST	Hepatocyte	Vesicular	Fetal brain	309	
			i ctai train	309	1
6586-7449	growth factor -	trafficking and			
	regulated tyrosine	signal transduction		}	
	kinase substrate.				
	Hrs (D84064)			-	
LYST	LIP10 (FIG. 11,		Heart	1	7
9037-9585	EST R17261)				·
	GBDR1	Glialblastoma cell	Keratinocyte	77	4
	(AF068195)	differentiation-	-		
		related protein			
	KB07	Similar to tyrosine	Heart	1	4
	(AF064606)	kinase		104	1

<sup>\*</sup> According to GenBank # U67615 (LYST), or GenBank or FIG. # in column 2 (LYST-IPs).

Table Ib. Protein interactions identified by reverse yeast two-hybrid screens using LYST baits

ACTIVATION	LYST-IP	IP function or % AA	HUMAN	START OF	# OF
DOMAIN	NAME	Identity [1],	BINDING	ISOLATES.	HITS
FRAGMENT. bp*	(GenBank #)	Similarity [S], #AA*	DOMAIN	bp*	PER
			LIBRARY		ISOLAT
					E
		1	F I I '	200	2
LYST	14-3-3 protein	Vesicular trafficking	Fetal brain	290	- 2
190-1056	(X56468)	and signal		356	2
		transduction			
	HSI	99% I, 100% S to	Fetal brain	465	2
	(X57346)	bovine 14-3-3 β;			
	(,,,,,,,,,,	signal transduction?			
	Calmodulin	Ca <sup>2*</sup> -binding:	Fetal brain	479	1
		vesicular trafficking,	i cui viuni	177	·
	(D45887)	signal transduction.			
	Estrogen	Signal transduction:	Fetal brain	939	3
	receptor-related	nuclear hormone			
	protein, hERR1	receptor,			
	(X51416)	transcription factor			
LYST	BMK1 alpha	Signal transduction:	Fetal brain	2121	1
2347-3213	kinase	Phosphorylation		2431	2
2347-3213	(1120725)			2431	-
	(U29725)				
LYST	14-3-3 protein	Vesicular trafficking	Fetal brain	290	I
4009-4821	(X56468)	and signal			
		transduction			
LYST	Importin β -	Nuclear protein	Fetal brain	1970	1
	subunit	transport; has ARM.			
6586-7449	(L38951)	HEAT repeats			
	Imogen 38	Langerhans β-cell	Fetal brain	473	1
	(Z68747)	antigen			
		(secr.granule/mitoch			
		ondr.)			
	L	L		L	

LYST

9037-9585

DiGeorge   Novel gene from the   Syndrome -1.   DGS critical region   (22q11)   (177566)   (22q11)   (21q11)   (21q11
DGS-1 (L77566)   (22q11)   (L77566)     (L77566)   (
(L77566)  KIAA0607 98% 1. 98% S to rat gene norbin (AB006461)  (AB011179)  LIP1. novel 66% 1. 85% S. 35. Fetal brain 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
KIAA0607   98% I. 98% S to rat norbin (AB006461)
gene   norbin (AB006461)
(AB011179)  LIP1, novel 66% I, 85% S, 35, Fetal brain I I I I I I I I I I I I I I I I I I I
LIP1. novel 66% 1, 85% S, 35, Fetal brain 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
(FIG. 2) human TCP10A protein (U03399)  L1P2, novel 40% I, 56% S, 110, Fetal brain I I (FIG. 3) bacterial ribosomal protein L17 (M26414) 21 I  L1P4, novel 62% I, 73% S, 89, Fetal brain 496 I (FIG. 5) human hnRNP-E2 (X78136)  L1P5, novel 35% I, 61% S, 131, Fetal brain 48 I
(FIG. 2) human TCP10A protein (U03399)  L1P2, novel 40% I, 56% S, 110, Fetal brain I (FIG. 3) bacterial ribosomal protein L17 (M26414) 21 I  L1P4, novel 62% I, 73% S, 89, Fetal brain 496 I (FIG. 5) human hnRNP-E2 (X78136)  L1P5, novel 35% I, 61% S, 131, Fetal brain 48 I
Description (U03399)   Description (U03399)
L1P2, novel 40% 1, 56% S, 110, Fetal brain 1 1 1
(FIG. 3) bacterial ribosomal protein L17 (M26414) 21 1  L1P4. novel 62% 1, 73% S, 89, Fetal brain 496 1  (FIG. 5) human hnRNP-E2 (X78136) 1  L1P5, novel 35% 1, 61% S, 131, Fetal brain 48 1
Protein L17
(M26414)  LIP4, novel 62% I, 73% S, 89, Fetal brain 496 I  (FIG. 5)  LIP5, novel 35% I, 61% S, 131, Fetal brain 48 I
LIP4. novel 62% I, 73% S, 89, Fetal brain 496 I human hnRNP-E2 (X78136)  LIP5, novel 35% I, 61% S, 131, Fetal brain 48 I
(FIG. 5) human hnRNP-E2 (X78136)  LIP5, novel 35% I, 61% S, 131, Fetal brain 48 1
(X78136)  LIP5, novel 35% I, 61% S, 131, Fetal brain 48 1
LIP5, novel 35% I, 61% S, 131, Fetal brain 48 1
LIP5, novel 35% I, 61% S, 131, Fetal brain 48 1
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[(FIG. 6)
(Z81130)
LIP6, novel 92% I, 93% S, 139, Fetal brain I 2
(FIG. 7) viral protein Ns2-3
(U43603)
LIP7, novel 30% I, 59% S, 81, Fetal brain I 2
human TCD104
(FIG. 8) protein (U03399)
LIP8, novel 63% 1, 63% S, 21, Fetal brain 1 2
(FIG. 9) rabbit KAP4L
protein (X80035)
LIP3. novel 72% I. 83% S. 172. Fetal brain 345 1
7.5
(FIG. 4) rat Zn tinger prot.Roaz (U92564)
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,

LYST	Atrophin-1	Causes DRPLA	Fetal brain	2659	
9502-10590	(U23851)	(Smith's disease);			
(BEACH domain)		vesicle transport?			
	Embryonic Fyn substrate I. Efs I (AB001466)	Signal transduction	Fetal brain	1664	2
	OPA-containing protein (AF071309)	Unknown function; no significant iden./similarity	Fetal brain	5262	1
	M4 protein (L03532)	RNA-binding protein	Fetal brain	782	ı
	LIP3, novel (FIG. 4)	72% I, 83% S. 172, rat Zn finger prot.Roaz (U92564)	Fetal brain	345	1
	LIP9, novel (FIG. 10)	61% I, 75% S, 180,  Xenopus etr-1 (U16800)	Fetal brain	138	1
LYST 10576-11611 (WD-40 domain)	Casein kinase II β-subunit (M30448)	Phosphorylation: vesicular trafficking, signal transd.	Fetal brain	60	•
	LIP6, novel (FIG. 7)	92% I, 93% S, 139, viral protein Ns2-3 (U43603)	Fetal brain	5	1

<sup>\*</sup> According to GenBank # U67615 (LYST), or GenBank or FIG. # in column 2 (LYST-IPs).

<sup>† #</sup>AA designates the number of amino acids in the region of LYST-IP, for which the % identity/similarity is shown

Table Ic. Protein interactions identified by reverse yeast two-hybrid screens using LYST-2

ACTIVATION	LYST-IP	LYST-IP	HUMAN	CTARGOR	
1		2131-11	HOMAN	START OF	# OF HITS
DOMAIN	NAME	FUNCTION	BINDING	ISOLATES.	PER
FRAGMENT, bp*	(GenBank #)		DOMAIN	bp*	ISOLATE
			LIBRARY		
LYST-2	HBF-G2		Fetal brain	1633	1
3-794	(X78202)				
LYST-2	14-3-3 protein	Vesicular	Fetal brain	356	1
774-1424	(X56468)	trafficking and signal transduction			
	XAP-4	Signal transduction	Fetal brain	1091	1
	(X79353)	(Rap GDP			
	,	dissociation			
		inhibitor)			
* According to	FIG. 1 /I VOT.	3) 6 5			

<sup>\*</sup> According to FIG. 1 (LYST-2), or GenBank # in column 2 (LYST-IPs).

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# EXAMPLE 2: VERIFICATION OF THE SPECIFICITY OF THE LYST:LYST-IP INTERACTIONS

To test for the specificity of bait:prey interaction, two general tests were first performed. In the first instance, YULH yeast cells were created that express the individual plasmids encoding the binding domain fusions of all LYST-interacting preys. The transformed haploid yeast cells were plated on SCS plates, grown overnight, and examined for growth. Cells that did not grow contained LYST interacting proteins that were not self-activating false positives. No growth was found for all proteins tested, confirming that they were not "self-activating" proteins, that is, these proteins require interaction with a second protein domain for a functional activation complex.

As an additional prey self-activation test, YULH and N106r haploid yeast, transformed with LYST-interacting binding domain or activating domain fusion protein preys, prespectively, were grown on SCS media, and a filter-lift beta-galactosidase assay was performed on the selected hapoid colonies. Colonies that did not turn blue contained non-self-activating prey. Only colonies passed both tests for prey self-activation were pursued. The specificity and reproducibility of interactions was evaluated in a matrix-mating test. Binding domain fusions with baits (forward screen) or prey (reverse screen) were re-transformed in N106r yeast and grown in YPAD medium at 30°C. A matrix mating test was performed by spreading baitcontaining haploid yeast over the surface of individual YPAD plates, followed by superimposing prey-containing haploids in a 12 x 8 matrix, and incubating the plates overnight at 30°C. Each plate contained positive (known protein interaction pairs) and negative controls. Resulting diploid colonies were replica-plated both on SCS plates (to select for activating and binding domain plasmids, diploids, and protein interactions ) and SC-Leu-Trp plates (that were used for a filter-lift beta-galactosidase assay). Baits and preys from LacZ+ diploids growing on SCS were sequenced. If the identity of a given bait-prey pair matched that obtained in an initial screen, the interaction was considered confirmed by the yeast two hybrid method.

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EXAMPLE 3: ANALYSIS OF THE SEQUENCES ENCODING LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10

# GENERAL PROCEDURE FOR THE ASSEMBLY AND IDENTITY SEARCHES OF THE SEQUENCES ENCODING EST

The human EST assemblies were done by using public available EST assembly databases such as the National Center for Biotechnology Information (N.C.B.I.) Blast 2.0 software program (Gish 1994-1997; Altschul *et al.*, 1990, *J. Mol. Biol. 215*: 403-410). Sequences that aligned with 95% or greater identity at the nucleic acid level over at least 30 nucleotides of their termini were utilized if the alignment resulted in 5' extension or 3' extension of the EST sequence. Once this first assembly was complete, the extended sequence was again subject to a Blast comparison to detect new homologies to the added extensions. The sequence was extended in both directions until new related sequences that allowed extension of the assembled sequence were no longer detected.

The assembled EST sequence was subjected to further searches using the BlastX 2.0 software program for the identification of protein coding regions by database similarity search (Gish 1994-1997; Gish and States 1993, *Nat. Genet. 3*: 266-272). This BlastX program uses translates the DNA sequence in all six reading frames and compares the translated protein sequence with those in protein databases. The statistical significance is estimated under the assumption that the equivalent of one entire reading frame in the query sequence codes for protein and that significant alignments will involve only coding reading frames. Sequences producing high-scoring segment pairs are shown.

Furthermore, the sequences were analyzed for open reading frames using proprietary software that translates the DNA sequence in all six reading frames of the (assembled) DNA sequence using the standard genetic code. The interacting ESTs were obtained from directionally-cloned libraries, and thus the direction of translation of the assembled EST is known as 5° to 3° and the open reading frame is also known. Within the translations obtained, the possible open reading frames ("ORFs") found in the correct frame was analyzed. Open reading frames greater than 50 amino acids following an initiator codon or an ORF with no initiator methionine encoded at the 5° end were determined to be possible protein products, and were compared to sequences in protein data bases using the BLASTP 2.0 software program

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(Gish 1994-1997; Altschul et al., 1990. J. Molec. Biol. 215: 403-410). Protein sequences with identities and similarities to known protein sequences are shown.

Further protein sequence analysis were performed after selecting a suitable open reading frame. The protein sequence was compared to previously characterized protein domains present in the BLOCKS and PRODOM motif databases (Bairoch, 1992, Nucleic Acids Research 20: 2013-2018; Henikoff and Henikoff 1991, Nucleic Acids Research 19-23: 6565-6512; Nakai and Kanehisa 1992, Genomics 14: 897-91; Wallace and Henikoff 1992, Cabios 8: 249-254). The BLIMPS software program found matches to entries in the BLOCKS. The BLOCKS analysis aligns similar sequence domains found in proteins and reveals the corresponding protein families. BLASTP found matches to entries in the PRODOM database. The PRODOM analysis displays the alignment that constitutes protein domains with high identities and similarities.

#### **EST ANALYSIS**

# EST cg50136.e7 (LIP1)

Four sequences (cg50136.e7, 493 nucleotides; cg50136.a2, 447 nucleotides; cg50136.e6, 443 nucleotides; and cg50136.f6, 392 nucleotides), 99% identical, were identified interacting with LYST (domain 6586-7449). These four sequences overlapped in a contig (wherein a contig is defined as a contiguous region of sequence identifiable by a series of assembled overlapping DNA clones) as 501 bp including human EST AA332916 (196 bp), nucleotides 266 to 461 of the contig were 99% identical to nucleotides 1 to 196 of AA332916. The 3'-end of this contig (282-501 bp) overlapped with two ESTs: 98% identical to nucleotides 1-128, 90% to nucleotides 339-455, and 71% to nucleotides 234-455 of the human EST AA452346 (455 nucleotides) and 97% identical to the human EST AA447226 (452 bp), both initially obtained from a human Soares total fetus (8-9 week) library (Hillier *et al.*, 1997). These ESTs overlapped with the initial sequences to form a 736 bp contig, EST cg50175.e7+, that could not be extended further. EST cg50175.e7+ showed 66% nucleotide identity to mouse T-complex protein Tcp-10c (GenBank accession No. M73506).

An open reading frame could be translated from nucleotides 3 to 593. The resulting 197 amino acids correspond to a C-terminal or core region of a novel protein, which was designated LIP-1. Amino acids 11-194 of LIP-1 show 61% identity and 78% similarity to the C-terminal region (amino acids 254-436) of mouse Tcp-10 protein (GenBank accession No. X58170). LIP-1 also shows 66% identity to human tcp10a protein (GenBank accession No. U03399)

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(nucleotides 89 to 194 of LIP-1 and nucleotides 286 to 390 of U03399). Therefore, LIP-1 represents a novel protein with homology to mouse and human Tcp-10 proteins. Interestingly, another LYST-interactant, LIP-7 (see later in this chapter), shows homology to the aminoterminal region of Tcp-10. The nucleotide and amino acid sequences of LIP-1 are shown in Figure 2.

#### LIP-2 (cg50136.a4)

Three sequences (cg50136.a4 - 446 bp, cg50136.d6 - 443 bp, cg50175.c9 - 380), 99% identical, interacted with LYST (6586-7449). These three sequences overlapped to form a 464 bp sequence. Nucleotides 1 to 454 of this sequence were 96% identical to nucleotides 389 to 842 of the human assembly hs6793\_0, 842 bp (CuraGen proprietary, unpublished). This assembly contains a number of published ESTs including nucleotides 1 to 698 from EST AI141700 (corresponding to nucleotides 112 to 781 in human assembly hs6793\_0), nucleotides 1 to 634 from EST W67725 (corresponding to nucleotides 22 to 675 in human assembly hs6793\_0), and nucleotides 1 to 604 from EST AA203533 (corresponding to nucleotides 98 to 692 in human assembly hs6793\_0). The overlap of the prey sequences and human assembly hs6793\_0 created a 850 contig that could not be further extended by database searches and was used for further analysis. This contig showed no significant similarity to any gene of known function by BlastN.

An open reading frame could be translated from nucleotides 1 to 552 of the contig. The resulting 184 amino acids correspond to a C-terminal or core region of a novel protein which was called LIP-2. Amino acids 24-133 of LIP-2 show 40% identity and 56% similarity to amino acids 2-117 of bacterial ribosomal protein L17 (GenBank accession No. M26414). Therefore, LIP-2 represents a novel human protein with similarity to ribosomal protein L17. The nucleotide and amino acid sequences of LIP-2 are shown in Figure 3.

#### LIP3 (EST cg50136.c10)

Two identical sequences (cg50136.c10 and cg50136.g10), interacting with LYST (9037-9585 and 9502-10590), showed significant identity to published ESTs. An extended expressed sequence of 1411 nucleotides was assembled from nucleotides 1 to 447 of human fetal lung EST W40354 (Hillier *et al.*, 1995, The WashU-Merck EST Project, direct submission to GenBank)nucleotides 1 to 382 from human endothelial cell EST AA446837 (Hillier *et al.*, 1997, WashU-Merck EST Project 1997, Unpublished) (corresponding to nucleotides 328 to 878 in the

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extended sequence). nucleotides 1 to 435 from cg50136.c10 (corresponding to nucleotides 345 to 781 in the extended sequence). nucleotides 1 to 422 from EST N49053 from a human multiple sclerosis library (Hillier *et al.*, 1995, The WashU-Merck EST Project, direct submission to GenBank) (corresponding to nucleotides 779 to 1201 in the extended sequence), and nucleotides 1 to 504 from EST Al097654 from a human brain library (corresponding to nucleotides 900 to 1410. The fragment interacting with LYST starts at nucleotide 345. The extended sequence of 1410 nucleotides was used for subsequent analysis. Nucleotides 213 to 599 of the extended expressed sequence showed 74% identity to nucleotides 3589 to 3975 of rat Olf-1/EBF associated Zn finger protein Roaz (GenBank accession No. U92564).

An open reading frame could be translated from nucleotides 1 to 594. These 198 amino acids represent a core or C-terminal region of a novel protein, which was designated LIP-3. Amino acids 26 to 197 of LIP-3 show 72% identity and 83% similarity to amino acids 1023 to 1185 of the rat Zn finger protein Roaz. Weaker homologies (40 to 50%) were seen to different zinc-finger proteins of several species. Interestingly, conserved zinc-finger domains exist in other proteins, e.g., protein kinase C and Hrs, that may be associated with LYST. Therefore, LIP-3 represents a novel human protein with homologies to rat Olf-1/EBF associated Zn finger protein Roaz. The nucleotide and amino acid sequences of LIP-3 are shown in Figure 4.

# LIP4 (EST cg50136.a7)

One identified prey sequence (cg50136.a7+, 243 nucleotides), interacting with LYST (6586-7449), was 97% identical to human EST W22541, starting at nucleotide 63 of the EST. This EST of 809 nucleotides was initially obtained from a human retina library (Macke *et al.*, 1996, direct submission to GenBank). EST W22541 contained cg50136.a7 and could be extended using ESTs AA009453 (505 nucleotides), AA482060 (457 nucleotides), AI193517 (314 nucleotides) and AA252768 (485 nucleotides) to a 921 nucleotide sequence. Nucleotides 627 to 397 of this assembly were 68% identical to nucleotides 843 to 1070 of human hnRNP-E1 mRNA (GenBank accession No. X78137).

Open reading frames could be translated from this sequence in all six frames. The longest ORF could be translated in frame +2 from nucleotide 71 to 898, 276 amino acids. As the protein translation depicted does not begin with an "ATG" initiator methionine codon, we surmise the protein sequence encoded by nucleotide residues 71 to 898, as shown, represents a C-terminal protein fragment, wherein the N-terminal protein sequence is not represented in the

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figure. This ORF was also identified to have the most similarity to known proteins using BlastX. Amino acids 77 to 165 of this 276 amino acid protein were 62% identical and 73% similar to human HNRNP-E2 protein (GenBank accession No. X78136). LIP-4 represents a novel, protein with similarity to human ribonuclear proteins. The nucleotide and amino acid sequences of LIP-4 are shown in Figure 5.

# LIP-5 (EST cg50175.c7)

One identified prey sequence (cg50175.c7, 450 nucleotides), interacting with LYST, was highly homologous to a number of known ESTs [98% identical to human cDNA clone AA173372 which is 480 nucleotides and was initially obtained from a Stratagene neuroepithelium (#937231) library (Hillier et al., 1997, WashU-NCI human EST Project, direct submission to GenBank), 96% identical to EST H19045 which is 521 nucleotides and was initially obtained from an infant brain library (Hillier et al., 1995, WashU-NCI human EST Project, direct submission to GenBank), 97% identical to EST R49709 which is 410 nucleotides and was initially obtained from an infant brain library (Hillier et al., 1995, WashU-NCI human EST Project, direct submission to GenBank), 97% identical to EST AA092322 which is 311 nucleotides and was initially obtained from a fetal heart library, 97% identical to EST Z36995 which is 381 nucleotides and was initially obtained from a human heart library, and 97% identical to EST Z60661 which is 242 nucleotides and was initially obtained from a human blood cell library]. These ESTs assembled into a 837 nucleotide sequence, and this sequence could not be extended further by database searches and was used for subsequent analysis. Nucleotides 585 to 257 of this sequence were 60% identical to nucleotides 1333 to 1662 of S. cerevisiae NOP77 gene for essential nucleolar protein (GenBank accession No. X76245).

An open reading frame starting at a methionine codon with a Kozak consensus sequence could be translated from nucleotides 31 to 696, indicating that this represents the full coding sequence of this gene. This coding sequence corresponds to a 221 amino acid protein, which was designated LIP-5. Amino acids 27 to 157 of LIP-5 are 35% identical and 61% similar to amino acids 315 to 461 of gene T23G11.7, C. Elegans. 885 aa (GenBank accession No. Z81130). Protein sorting and signal peptide prediction programs indicate that there is no signal peptide in this protein. Using BLOCKS protein domain analysis, LIP-5 has a high probability of containing a phosphorylase pyridoxal-phosphate attachment site (strength - 1682, score - 1067). LIP-5

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represents a novel human protein. The nucleotide and amino acid sequences of LIP-5 are shown in Figure 6.

# LIP-6 (EST cg50136.f9)

Four identified sequences (cg50136.g5 - 360 nucleotides. cg50136.g5 - 304 nucleotides. cg50136.f9 - 486 nucleotides, and cg50138.f2 - 160 bp) interacting with LYST (two were identified to interact with 10576-11611 and two were identified to interact with 6586-7449). These four sequences formed a contig identical to the longest sequence, cg50136.f9. Nucleotides 166 to 486 of cg50136.f9 were 99% identical to nucleotides 1 to 320 of EST AA460131 (588 nucleotides; Hillier *et al.*, 1997, WashU-NCI human EST Project, direct submission to GenBank). This sequence was obtained from a human total fetus (9 weeks) cDNA library.

A longer sequence was assembled from nucleotides 1-486 of cg50136.f9 and nucleotides 321-588 of AA460131. The extended sequence of 754 nucleotides was used for subsequent analysis. The extended sequence showed similarity to several viral nonstructural proteins: nucleotides 2 to 488 showed 83% identity to nucleotides 170 to 653 of the nonstructural protein Ns2-3 of a border disease virus strain (pestivirus type 3) (GenBank accession No. U43603) and nucleotides 43 to 406 showed 90% identity to nucleotides 17 to 378 of proteins p54, p80 and p125 of the bovine viral diarrhea virus (GenBank accession No. Z54331).

An open reading frame could be translated from nucleotides 2 to 583, 194 amino acids. An open reading frame with a Kozak consensus start methionine could be translated from nucleotides 11 to 583, 191 amino acids. These 191 amino acids represent a novel protein, which was called LIP-6. Amino acids 3 to 141 of LIP-6 show 92% identity and 93% similarity to amino acids 64 to 198 of the viral (pestivirus type 3) nonstructural protein Ns2-3 (GenBank accession No. U43603). Amino acids 28 to 132 are 97% homologous to amino acids 22 to 126 of the nonstructural protein p125 of the bovine viral diarrhea virus (GenBank accession No. Z54331). BLOCKS analysis searching for similarities to known protein families showed some similarity to 14-3-3 proteins (amino acids 15 to 65: identities are shown in capital letters): (N-ter) Ef1SK1QDdLKeamntmmCSRcQGkhRrFemdrEpksaRYcAEcnrlhpAE (C-ter) [SEQ ID NO:45]. Therefore, LIP-6 represents a novel human protein with similarity to viral nonstructural proteins. The nucleotide and amino acid sequences of LIP-6 are shown in Figure 7.

# 128 LIP-7 (EST cg50136.a5)

Two identified sequences [cg50136a5 (445 bases) and cg50136c4 (451 bases)] interact with LYST (6586 - 7449). Aligns with EST aa627098 (NCI\_CGAP\_Br2 Hs cDNA 499bp), nucleotides 449 to 155 of cg50136.a5.b align with nucleotides 204 to 498 of aa627098 - 92% identity over 295 bp. Alignment created a 653bp contig. No significant identity to any other published sequences could be identified. Nucleotides 627 to 227 were 62% identical to nucleotides 342 to 738 of the mycoplasma hominis vaa (p50/adhesin) gene (GenBank accession No. AJ001653).

An open reading frame could be translated from nucleotides 2 to 586. These 195 amino acids correspond to a C-terminal or core region of a novel protein, which was called LIP-7. Amino acids 59 to 139 of LIP-7 showed 30% identity and 59% similarity to amino acids 162 to 404 of the human T-complex protein 10A (TCP10A) (GenBank accession No. U03399). Amino acids 10 to 149 of LIP-7 showed 29% identity and 54% similarity to amino acids 4039 to 4461 of the human trichohyalin (TRHY) gene (GenBank accession No. L09190). Amino acids 6 to 186 of LIP-7 showed 28% identity and 51% similarity to amino acids 2387 to 2890 of the human neuronal kinesin heavy chain (GenBank accession No. U06698).

Other homologies (~50%) were found to several cytoskeletal proteins (e.g. myosin I isoform, kinesin precursor, cytokeratin, caldesmon, NUF1, tropomyosin, neurofilament protein, kinesin-like protein KIF1, myosin like protein MLP1, troponin T) and to proteins involved in vesicular transport (intracellular protein transport protein, nuclear fusion protein Bik1, synaptonemal complex protein). BLOCKS analysis showed homologies to postsynaptic proteins, gas vesicles protein, clusterin proteins, clathrin light chain proteins, tropomyosin protein and intermediate filament protein. Therefore, LIP-7 represents a novel protein with homologies to the amino-terminal region of Tcp-10 and with homologies to cytoskeletal and vesicular transport proteins. It is interesting that the LYST-interactant LIP-1 shows similarity to the C-terminal region of Tcp-10. The nucleotide and amino acid sequences of LIP-7 are shown in Figure 8.

# LIP-8 (EST cg50136.d2)

Two identified sequences (cg50136c6 and cg50136d2), interacting with LYST (6586-7449), showed no significant homologies to published sequences. The interacting sequence of 386 nucleotides, named cg50136d2±, was used for further analysis. Nucleotides 27 to 244 of

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cg50136d2+ were 69% homologous to nucleotides 1115 to 1335 of the human RNA for the cellular oncogene c-fes (GenBank accession No. X52192).

An open reading frame could be translated from nucleotides 3 to 359. These 119 amino acids correspond to a C-terminal or core region of a novel protein, which was called LIP-8. Only weak homologies to known proteins could be found. The strongest Blast score, 63% identity and 63% similarity using BLASTP, was found between amino acids 36 to 56 in LIP-8 and amino acids 81 to 102 in a *Oryctolagus cuniculus* (rabbit) cysteine rich hair keratin associated protein (GenBank accession No. X80035). LIP-8 represents a novel protein. The nucleotide and amino acid sequences of LIP-8 are shown in Figure 9.

# LIP-9 (EST cg50175.c11)

One identified sequence (cg50175.c11. 324 nucleotides), interacting with LYST (9502-10590), showed significant identity to published expressed sequences. Nucleotides 2 to 324 of cg50175.c11 were 96% identical to nucleotides 73 to 394 of the expressed sequence H51347 (428 nucleotides; Hillier *et al.*, 1995, WashU-NCI human EST Project, direct submission to GenBank), obtained from a human adult brain cDNA library and nucleotides 324 to 43 of cg50175.c11 were 98% identical to nucleotides 188 to 468 of the expressed sequence AA628560 (468 nucleotides; Hillier *et al.*, 1997, WashU-NCI human EST Project, direct submission to GenBank), obtained from a human total fetus cDNA library. These two ESTs assembled into a 418 nucleotide sequence that could be extended further using ESTs AA041634 (949 nucleotides) and H20082 (322 nucleotides), creating a 1237 nucleotide sequence. Nucleotides 1018 to 274 were 73% identical to human CAGH4 mRNA (GenBank accession No. U80746) and nucleotides 1088 to 528 showed 69% identity to nucleotides 523 to 1085 of Xenopus laevis elav-type ribonucleoprotein etr-1 (GenBank accession No. U16800).

Open reading frames could be translated from this sequence in all six frames. The longest ORF could be translated in frame 3 from nucleotide 78 to 716, 213 amino acids.

This ORF was also identified to have the most similarity to known proteins using BlastX. Amino acids 7 to 186 showed 61% identity and 75% similarity to amino acids 59 to 248 of Xenopus laevis elav-type ribonucleoprotein etr-1 (GenBank accession No. U16800) and 44% identity and 61% similarity to the human etr-3 protein (GenBank accession No. U69546). LIP-9 represents a novel human protein with similarity to ribonucleoproteins. The nucleotide and amino acid sequences of LIP-9 are shown in Figure 10.

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# LIP-10 (EST cg152106.fl1)

Seven sequences (cg152082\_d3, 273 nucleotides: cg152106\_f11, 392 nucleotides; cg152106\_f9, 360 nucleotides: cg 152106\_g1, 247 nucleotides: cg152106\_g2, 369 nucleotides: cg152106\_g4, 351 nucleotides: and cg152106\_g5, 321 nucleotides). 99% identical, were identified interacting with LYST (9037-9585). These seven sequences overlapped in a contig that was 392 bp. This contig included EST R17261 (373 bp) initially obtained from an infant brain library (Hillier *et al.*, 1995, WashU-NCI human EST Project, direct submission to GenBank); nucleotides 18 to 365 of the contig were 91% identical to nucleotides 1 to 348 of EST R17261. This contig cg152106\_f11+ could not be extended further. Contig cg152106\_f11+ showed 60% nucleotide identity and 60% nucleotide similarity to cat glyceraldehyde-3-phosphate dehydrogenase mRNA, partial cds (GenBank accession No. AF054608).

An open reading frame could be translated in the +1 frame from nucleotides 1 to 390 (130 amino acids). As the protein translation depicted does not begin with an "ATG" initiator methionine codon, we surmise the protein sequence encoded by nucleotide residues 78 to 716, as shown, represents a C-terminal protein fragment, wherein the N-terminal protein sequence is not represented in the figure. The best match for this ORF using BlastX to search for protein sequence similarity was to tyrosine kinase VEGFR-3 from chicken (GenBank accession No. AF041795). Amino acids 1 to 33 of Contig cg152106\_f11+ were 40% identical and 65% similar to amino acids 33 to 64 of chicken tyrosine kinase VEGFR-3. The nucleotide and amino acid sequences of LIP-10 are shown in Figure 11.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

#### **EQUIVALENTS**

From the foregoing detailed description of the specific embodiments of the invention, it should be apparent that compositions and methods of use for the lysosomal proteins LYST or

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LYST-2 and their interacting proteins have been described. Although particular embodiments have been disclosed herein in detail, this has been done by way of example for purposes of illustration only, and is not intended to be limiting with respect to the scope of the appended claims which follow. In particular, it is contemplated by the inventor that various substitutions, alterations, and modifications may be made to the invention without departing from the spirit and scope of the invention as defined by the claims. For instance, the choice of fragment length, or recombinant methodology is believed to be a matter of routine for a person of ordinary skill in the art with knowledge of the embodiments described herein.

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#### WHAT IS CLAIMED IS:

- 1. A purified complex of LYST protein and a LYST-IP protein in which the LYST-IP protein is selected from the group consisting of 14-3-3 protein. HS1 protein. Hrs. BMK1. KB07. Efs. OS9. casein kinase II beta SU, calmodulin. troponin I. importin beta. Fte-1. hERRa1, imogen 38. atrophin-1. norbin, HBF-G2. DGS-I. GBDR1. OPA containing protein. M4 protein, LIP1, LIP2, LIP3. LIP4. LIP5, LIP6, LIP7. LIP8, LIP9. or LIP10.
- 2. A purified complex of LYST-2 protein and a LYST-IP protein in which the LYST-IP protein is selected from the group consisting of XAP4, HBF-G2, and 14-3-3 protein.
- 3. The purified complex of claim 1 or 2 in which the proteins are human proteins.
- 4. A purified complex selected from the group consisting of a complex of a derivative of a LYST or LYST-2 and a LYST-IP protein, a complex of a LYST protein and a derivative of a LYST-IP, and a complex of a derivative of LYST or LYST-2 and a derivative of a LYST-IP; in which the derivative of the LYST or LYST-2 protein is able to form a complex with a wild-type LYST-IP protein and the derivative of the LYST-IP is able to form a complex with a wild-type LYST or LYST-2 protein; and in which the LYST-IP is selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1. KB07. Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERRa1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10, and LIP10.
- 5. The purified complex of claim 4 in which the derivative of the LYST or LYST-2 protein and/or the LYST-IP protein is fluorescently labeled.
- 6. A chimeric protein comprising a fragment of a LYST or LYST-2 protein consisting of at least 6 amino acids fused via a covalent bond to a fragment of a LYST-IP protein consisting of at least 6 amino acids.

- 7. The chimeric protein of claim 6 in which the fragment of the LYST or LYST-2 protein is a fragment capable of binding the LYST-IP protein and in which the fragment of the LYST-IP protein is a fragment capable of binding the LYST or LYST-2 protein.
- 8. The chimeric protein of claim 7 in which the fragment of the LYST or LYST-2 protein and the fragment of the LYST-IP protein form a LYST:LYST-IP complex.
- 9. An antibody which immunospecifically binds the complex of claim 1 or 2 or a fragment or derivative of said antibody containing the binding domain thereof.
- 10. The antibody of claim 9 which does not immunospecifically bind a LYST or LYST-2 protein or a LYST-IP protein that is not part of a LYST-IP complex.
- 11. An isolated nucleic acid or an isolated combination of nucleic acids comprising a nucleotide sequence encoding a LYST or LYST-2 protein and a nucleotide sequence encoding a LYST-IP protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38. atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9, and LIP10.
- 12. The isolated nucleic acid or isolated combination of nucleic acids of claim 11 which are nucleic acid vectors.
- 13. The isolated nucleic acid or isolated combination of nucleic acids of claim 12 in which the LYST or LYST-2 protein coding sequence and the LYST-IP protein coding sequence are operably linked to a promoter.
- 14. An isolated nucleic acid that comprises a nucleotide sequence encoding the chimeric protein of claim 8.

- 15. A cell containing a nucleic acid of claim 11, which nucleic acid is recombinant.
- 16. A cell containing a nucleic acid of claim 13, which nucleic acid is recombinant.
- 17. A recombinant cell containing a nucleic acid of claim 14, which nucleic acid is recombinant.
- 18. A purified protein selected from the group consisting of LYST-2, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, and LIP9.
  - 19. The protein of claim 18 which is a human protein.
- 20. The protein of claim 19 which comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24.
- 21. A purified protein encoded by a nucleic acid hybridizable to the inverse complement of a DNA having a nucleotide sequence consisting of a portion of the nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21 and SEQ ID NO:23.
- 22. A purified derivative or analog of the protein of claim 18, which derivative or analog can bind LYST or LYST-2.
- 23. The derivative or analog of claim 22 which is able to be bound by an antibody directed against a protein selected from the group consisting LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, and LIP9.

- 24. A purified fragment of the protein of claim 18, which fragment comprises an at least 6 amino acid portion of said protein.
- 25. A purified protein comprising an amino acid sequence that has at least 60% identity to the protein of claim 18. in which the percentage identity is determined over an amino acid sequence of identical size to said protein of claim 18.
- 26. A chimeric protein comprising a fragment of the protein of claim 18, said fragment consisting of at least 6 amino acids of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, and LIP9, fused via a covalent bond to an amino acid sequence of a second protein, in which the second protein is not said protein of claim 18.
- 27. An antibody which immunospecifically binds the protein of claim 18, or a fragment or derivative of said antibody containing the binding domain thereof.
- 28. An isolated nucleic acid comprising a nucleotide sequence encoding the protein of claim 18.
- 29. An isolated nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23.
- 30. An isolated nucleic acid hybridizable to the inverse complement of a DNA having a nucleotide sequence consisting of a portion of the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9. SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23.
- 31. An isolated nucleic acid comprising a portion of the nucleotide sequence of SEQ.ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11. SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, and SEQ ID NO:23, said nucleic acid comprising an at least 10 nucleotide sequence.

- 32. A cell containing the nucleic acid of claim 28, which nucleic acid is recombinant.
- 33. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the complex of claim 1 or 2; and a pharmaceutically acceptable carrier.
- 34. The pharmaceutical composition of claim 33 in which the proteins are human proteins.
- 35. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the complex of claim 4; and a pharmaceutically acceptable carrier.
- . 36. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the chimeric protein of claim 6; and a pharmaceutically acceptable carrier.
- 37. A pharmaceutical composition of comprising a therapeutically or prophylactically effective amount of the chimeric protein of claim 7; and a pharmaceutically acceptable carrier.
- 38. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the antibody of claim 9 or a fragment or derivative of said antibody containing the binding domain thereof; and a pharmaceutically acceptable carrier.
- 39. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the antibody of claim 10 or a fragment or derivative of said antibody containing the binding domain thereof: and a pharmaceutically acceptable carrier.

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- 40. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the nucleic acid or combination of nucleic acids of claim 11: and a pharmaceutically acceptable carrier.
- 41. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the isolated nucleic acid of claim 14; and a pharmaceutically acceptable carrier.
- 42. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the recombinant cell of claim 15; and a pharmaceutically acceptable carrier.
- 43. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the protein of claim 16; and a pharmaceutically acceptable carrier.
- 44. The pharmaceutical composition of claim 42 in which, the proteins comprise the amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24.
- 45. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of the antibody of claim 27 or a fragment or derivative of said antibody containing the binding domain thereof; and a pharmaceutically acceptable carrier.
- 46. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a nucleic acid comprising a nucleotide sequence encoding the protein of claim 18: and a pharmaceutically acceptable carrier.
- 47. A pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a cell containing the recombinant nucleic acid of claim 28: and a pharmaceutically acceptable carrier.

- 48. A method of producing a complex of a LYST or LYST-2 protein and a LYST-IP protein comprising growing a recombinant cell containing the nucleic acid of claim 11 such that the encoded LYST or LYST-2 and LYST-IP proteins are expressed and bind to each other, and recovering the expressed complex of the LYST or LYST-2 protein and the LYST-IP protein.
- 49. A method of producing a protein selected from the group consisting of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, and LIP9 comprising growing a cell containing a recombinant nucleic acid encoding said protein such that the encoded protein is expressed, and recovering the expressed protein.
- 50. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a complex of LYST or LYST-2 protein and a LYST-IP protein, in which the LYST-IP is selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, HERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10, in a subject comprising measuring the level of said complex, RNA encoding the LYST or LYST-2 and LYST-IP proteins, or functional activity of said complex in a sample derived from the subject, in which an increase or decrease in the level of said complex, said RNA encoding LYST or LYST-2 and LYST-IP, or functional activity of said complex in the sample, relative to the level of said complex, said RNA encoding LYST or LYST-2 and LYST-IP or functional activity of said complex found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.
- 51. A method of diagnosing or screening for the presence of or a predisposition for developing a disease or disorder characterized by an aberrant level of a protein or RNA selected from the group consisting of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 protein or RNA in a subject comprising measuring the level of said protein, said RNA or the functional activity of said protein in a sample derived from the subject, in which an increase

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or decrease in the level of said protein, said RNA, or said functional activity in the sample, relative to the level of said protein, said RNA, or said functional activity found in an analogous sample not having the disease or disorder or a predisposition for developing the disease or disorder, indicates the presence of the disease or disorder or a predisposition for developing the disease or disorder.

- 52. A kit comprising in one or more containers a substance selected from the group consisting of a complex of LYST or LYST-2 and a LYST-IP, an antibody against said complex, nucleic acid probes capable of hybridizing to RNA of LYST or LYST-2 and RNA of said LYST-IP, or pairs of nucleic acid primers capable of priming amplification of at least a portion of a gene for LYST or LYST-2 and a gene for said LYST-IP, in which said LYST-IP is selected from the group consisting of 14-3-3 protein. HS1 protein. Hrs. BMK1, KB07. Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10.
- 53. A method of treating or preventing a disease or disorder involving aberrant levels of a complex of LYST or LYST-2 and LYST-IP, in which the LYST-IP is selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10 in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule or molecules that modulate the function of said complex.
- 54. The method of claim 53 in which said disease or disorder involves decreased levels of said complex and said molecule or molecules promote the function of the complex of LYST or LYST-2 and LYST-IP and are selected from the group consisting of a complex of LYST or LYST-2 and LYST-IP; a derivative or analog of a complex of LYST or LYST-2 and LYST-IP, which complex is more stable or more active than the wild type complex: nucleic acids encoding the LYST or LYST-2 and LYST-IP proteins; and nucleic acids encoding

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a derivative or analog of LYST or LYST-2 and LYST-IP that form a complex that is more stable or more active than the wild type complex.

- 55. The method of claim 53 in which said disease or disorder involves increased levels of said complex and said molecule or molecules inhibit the function of said complex and are selected from the group consisting of an antibody against said complex or a fragment or derivative thereof containing the binding region thereof; LYST or LYST-2 and LYST-IP antisense nucleic acids; and nucleic acids comprising at least a portion of a LYST or LYST-2 and a LYST-IP gene into which a heterologous nucleotide sequence has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the LYST or LYST-2 and LYST-IP genes, in which the LYST or LYST-2 and the LYST-IP gene portions flank the heterologous sequences so as to promote homologous recombination with genomic LYST or LYST-2 and LYST-IP genes.
- 56. A method of treating or preventing a disease or disorder involving an aberrant level of a LYST-IP selected from the group consisting of LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9, and LIP10 in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of said LYST-IP.
- 57. The method of claim 56 in which said disease or disorder involves a decreased level of the LYST-IP and said molecule promotes the function of the LYST-IP and is selected from the group consisting of the LYST-IP protein, derivative or analog of the LYST-IP that is active in binding LYST or LYST-2, a nucleic acid encoding the LYST-IP protein, and a nucleic acid encoding a derivative or analog of the LYST-IP that is active in binding LYST or LYST-2.
- 58. The method of claim 56 in which said disease or disorder involves an increased level of the LYST-IP and said molecule inhibits the LYST-IP function and is selected from the group consisting of an anti-LYST-IP antibody or a fragment or derivative thereof containing the binding region thereof, a LYST-IP antisense nucleic acid, and a nucleic acid comprising at least a portion of the LYST-IP gene into which a heterologous nucleotide sequence

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has been inserted such that said heterologous sequence inactivates the biological activity of the at least a portion of the LYST-IP gene, in which the LYST-IP gene portion flanks the heterologous sequence so as to promote homologous recombination with a genomic LYST-IP gene.

- LYST-IP selected from the group consisting of 14-3-3 protein, HS1 protein. Hrs. BMK1, KB07, Efs. XAP-4. OS9. casein kinase II beta SU. calmodulin. troponin I, importin beta. Fte-1, hERR1, imogen 38. atrophin-1, norbin, HBF-G2. DGS-I. GBDR1, OPA containing protein. M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7. LIP8, LIP9 and LIP10, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing an atopic disorder comprising contacting cultured cells that exhibit an indicator of an atopic disorder in vitro with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing atopic disorder.
- LYST-IP selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs. BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38. atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein. M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9 and LIP10, or a derivative of said complex. or a modulator of the activity of said complex for activity in treating or preventing an autoimmune disorder comprising contacting cultured cells that exhibit an indicator of a autoimmune disorder in vitro with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing autoimmune disorder.
- 61. A method for screening a purified complex of LYST or LYST-2 and a LYST-IP selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs. BMK1, KB07, Efs. XAP-4. OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imagen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein,

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LIP1. LIP2. LIP3. LIP4. LIP5. LIP6. LIP7. LIP8. LIP9 and LIP10 . or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing a neurodegenerative disease comprising contacting cultured cells that exhibit an indicator of a neurodegenerative disease in vitro with said complex. derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing neurodegenerative disease.

- 62. A method of screening a purified complex of LYST or LYST-2 and a LYST-IP selected from the group consisting of 14-3-3 protein. HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10 or a derivative of said complex, or a modulator of the activity of said complex for anti-cancer activity comprising measuring the survival or proliferation of cells from a cell line which is derived from or displays characteristics associated with malignant disorder, which cells have been contacted with the complex, derivative, or modulator; and comparing the survival or proliferation in the cells contacted with the complex, derivative or modulator with said survival or proliferation in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has anti-tumor activity.
- 63. A method of screening a purified complex of LYST or LYST-2 and a LYST-IP selected from the group consisting of 14-3-3 protein. HS1 protein, Hrs, BMK1, KB07. Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1. OPA containing protein, M4 protein. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a derivative of said complex, or a modulator of the activity of said complex for anti-cancer activity by a method comprising administering the complex, derivative or modulator to a test animal, which test animal has a tumor, or which test animal does not have a tumor and is subsequently challenged with tumor cells or tumorigenic agents; and measuring tumor growth or regression in said test animal, wherein decreased tumor growth or increased tumor regression or prevention of tumor

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growth in test animals administered said complex, derivative or modulator compared to test animals not so administered indicates that the complex, derivative or modulator has anti-cancer activity.

- 64. A method for screening a purified complex of LYST or LYST-2 and a LYST-IP selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs. BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta. Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing pigmentation disorders comprising contacting cultured cells that exhibit an indicator of an pigmentation reaction in vitro with said complex, derivative or modulator; and comparing the level of said indicator in the cells contacted with the complex, derivative, or modulator with said level of said indicator in cells not so contacted, wherein a lower level in said contacted cells indicates that the complex, derivative or modulator has activity in treating or preventing pigmentation disorder or pigmentation disorders-associated diseases.
- LYST-IP selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta. Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing platelet dysfunction, including bleeding tendency, comprising administering said complex, derivative or modulator to a test animal, which test animal exhibits an platelet dysfunction, or which test animal does not exhibit an platelet dysfunction and is subsequently challenged with an agent that elicits an platelet dysfunction; and measuring the change in the platelet dysfunction after the administration of said complex, derivative or modulator, wherein a reduction in said platelet dysfunction or prevention of said platelet dysfunction indicates that the complex, derivative or modulator has activity in treating or preventing platelet dysfunction or platelet dysfunction-associated disease.

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- 66. A method for screening a purified complex of LYST or LYST-2 and a LYST-IP selected from the group consisting of 14-3-3 protein. HS1 protein. Hrs. BMK1, KB07, Efs. XAP-4. OS9. casein kinase II beta SU. calmodulin. troponin I, importin beta. Fte-1, hERR1, imogen 38. atrophin-1, norbin, HBF-G2. DGS-I. GBDR1. OPA containing protein. M4 protein. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a derivative of said complex, or a modulator of the activity of said complex for activity in treating or preventing viral infection and associated diseases comprising administering said complex, derivative or modulator to a test animal, which test animal exhibits symptoms of a viral infection or which test animal is predisposed to develop symptoms of a viral infection; and measuring the change in said symptoms of the viral infection after administration of said complex, derivative, or modulator, wherein a reduction in the severity of the symptoms of the viral infection or prevention of the symptoms of the viral infection indicates that the complex, derivative or modulator has activity in treating or preventing viral infection.
- the formation of a complex of LYST or LYST-2 and LYST-IP, in which said LYST-IP is selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10 comprising measuring the levels of said complex formed from LYST or LYST-2 and LYST-IP proteins in the presence of said molecule under conditions conducive to formation of the complex; and comparing the levels of said complex with the levels of said complex that are formed in the absence of said molecule, wherein a lower or higher level of said complex in the presence of said molecule indicates that the molecule modulates formation of said complex.
- 68. A recombinant non-human animal in which both an endogenous LYST or LYST-2 gene and an endogenous LYST-IP gene selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6,

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LIP7. LIP8. LIP9 and LIP10 have been deleted or inactivated by homologous recombination or insertional mutagenesis of said animal or an ancestor thereof.

- 69. A recombinant non-human animal containing both a LYST or LYST-2 gene and a LYST-IP gene selected from the group consisting of 14-3-3 protein. HS1 protein. Hrs, BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, in which the LYST or LYST-2 gene is under the control of a promoter that is not the native LYST or LYST-IP gene promoter.
- 70. A recombinant non-human animal containing a transgene comprising a nucleic acid sequence encoding the chimeric protein of claim 8.
- 71. A recombinant non-human animal containing a transgene comprising the nucleotide sequence of SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53.
- 72. A method of modulating the activity or levels of LYST or LYST-2 by contacting a cell with, or administering an animal expressing a LYST or LYST-2 gene, a protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a nucleic acid encoding said protein or an antibody that immunospecifically binds said protein or a fragment or derivative of said antibody containing the binding domain thereof.
- 73. A method of modulating the activity or levels of a protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imagen 38.

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atrophin-1, norbin. HBF-G2. DGS-I. GBDR1. OPA containing protein. M4 protein. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9 and LIP10 by contacting a cell with, or administering an animal expressing a gene encoding said protein. LYST, or LYST-2, or a nucleic acid encoding LYST, or LYST-2, or an antibody that immunospecifically binds LYST or LYST-2, or a fragment or derivative of said antibody containing the binding domain thereof.

- 74. A method of modulating the activity or levels of a complex of LYST or LYST-2 and a protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs. BMK1, KB07, Efs, XAP-4. OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38. atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, by contacting a cell with, or administering an animal expressing and forming said complex, a molecule that modulates the formation of said complex.
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- 76. The method of claim 74 wherein said contacting is carried out by administering the candidate molecules to the recombinant non-human animal of claim 68.
- 77. The method of claim 75 wherein said contacting is carried out in vitro; and LYST or LYST-2, said protein, and said candidate molecules are purified.

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- 58. A method for screening a derivative or analog of LYST or LYST-2 for biological activity comprising contacting said derivative or analog of LYST or LYST-2 with a protein selected from the group consisting of 14-3-3 protein. HS1 protein. Hrs. BMK1, KB07, Efs. XAP-4. OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta. Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10; and detecting the formation of a complex between said derivative or analog of LYST or LYST-2 and said protein; wherein detecting formation of said complex indicates that said derivative or analog of LYST or LYST-2 has biological activity.
- the group consisting 14-3-3 protein, HS1 protein. Hrs, BMK1, KB07, Efs, XAP-4. OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5. LIP6, LIP7, LIP8, LIP9 and LIP10 for biological activity comprising contacting said derivative or analog of said protein with LYST or LYST-2; and detecting the formation of a complex between said derivative or analog of said protein and LYST or LYST-2; wherein detecting the formation of said complex indicates that said derivative or analog of said protein has biological activity.
- characterized by an aberrant level of a complex of LYST or LYST-2 protein and a LYST-IP protein in a subject administered said treatment for said disease or disorder comprising measuring the level of said complex, RNA encoding the LYST or LYST-2 and LYST-IP proteins. or functional activity of said complex in a sample derived from said subject wherein said sample is taken from said subject after the administration of said treatment and compared to (a) said level in a sample taken from said subject prior to the administration of the treatment or (b) a standard level associated with the pretreatment stage of the disease or disorder, in which the change, or lack of change in the level of said complex, said RNA encoding LYST or LYST-2 and LYST-IP, or functional activity of said complex in said sample taken after the administration of said treatment relative to the level of said complex, said RNA encoding LYST or LYST-2 and LYST-IP or functional activity of said complex, said RNA encoding LYST or LYST-2 and LYST-IP or functional activity of said complex in said sample taken before the administration of

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said treatment or to said standard level indicates whether said administration is effective for treating said disease or disorder.

- 81. A method of treating or preventing atopic disorders in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting 14-3-3 protein, HS1 protein, Hrs, BMK1. KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a combination of any one or more of the foregoing.
- 82. A method of treating or preventing autoimmune disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1, KB07, Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a combination of any one or more of the foregoing.
- 83. A method of treating or preventing neurodegenerative disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1. KB07. Efs, XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta. Fte-1. hERR1, imogen 38, atrophin-1. norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein. M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8. LIP9 and LIP10, or a combination of any one or more of the foregoing.
- 84. A method of treating or preventing cancer or a hyperproliferative disorder in a subject comprising administering to a subject in which such treatment or prevention is

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desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting of 14-3-3 protein. HS1 protein. Hrs, BMK1. KB07. Efs. XAP-4, OS9. casein kinase II beta SU, calmodulin. troponin I, importin beta. Fte-1. hERR1. imogen 38, atrophin-1. norbin. HBF-G2, DGS-I. GBDR1. OPA containing protein. M4 protein. LIP1, LIP2, LIP3. LIP4, LIP5, LIP6, LIP7. LIP8. LIP9 and LIP10, or a combination of any one or more of the foregoing.

- associated disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting of 14-3-3 protein, HS1 protein. Hrs. BMK1, KB07, Efs, XAP-4. OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a combination of any one or more of the foregoing.
- disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1. KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a combination of any one or more of the foregoing.
- 87. A method of treating or preventing viral infection or an associated disease in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule that modulates the function of a complex of LYST or LYST-2 and a LYST-IP protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs, BMK1. KB07. Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2,

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DGS-I, GBDR1, OPA containing protein, M4 protein, LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, or a combination of any one or more of the foregoing.

88. A purified fragment of a protein selected from the group consisting of 14-3-3 protein, HS1 protein, Hrs. BMK1, KB07, Efs. XAP-4, OS9, casein kinase II beta SU, calmodulin, troponin I, importin beta, Fte-1, hERR1, imogen 38, atrophin-1, norbin, HBF-G2, DGS-I, GBDR1, OPA containing protein, M4 protein. LIP1, LIP2, LIP3, LIP4, LIP5, LIP6, LIP7, LIP8, LIP9 and LIP10, which fragment binds LYST or LYST-2.

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1	ATACTTCTGA	TGTAAAGGAA	CTAATTCCAG	AGTTCTACTA	CCTACCAGAG	50
	ThrSerAs	pValLysGlu	LeuIleProG	luPheTyrTy	rLeuProGlu	15
					AAGATGAAGT	
15	MetPheValA	snSerAsnGl	yTyrAsnLeu	${\tt GlyValArgG}$	luAspGluVa	31
					CCTGAAGACT	
32	1ValValAsn	${\tt AspValAspL}$	euProProTr	pAlaLysLys	ProGluAspP	48
					TTCTTGCCAA	
49	heValArgIl	eAsnArgMet	AlaLeuGluS	erGluPheVa	lSerCysGln	65
					a100100101	250
					GAGGACCAGA	
65	LeuHisGlnT	rplleAspLe	ullepnedry	TYTLYSGINA	rgGlyProGl	9.T
			mmmmmc » cm»	COPCACTTAT	GAAGGCTCTG	300
					GluGlySerV	
82	UAIAVAIAIG	ATaleuasny	grenenrary	Ineginity.	0140175017	,,
201	ጥርል እርርጥርርል	тастатсаст	GATCCTGTGC	TCAGGGAGGC	CATGGAGGCA	350
90	al AgnI.euAg	nSerIleThr	AspProValL	euArgGluAl	aMetGluAla	115
	42110111104110	<b>,,,,,,</b>		_		
351	CAGATACAGA	ACTTTGGACA	GACGCCATCT	CAGTTGCTTA	TTGAGCCACA	400
					leGluProHi	
401	TCCGCCTCGG	AACTCTGCCA	TGCACCTGTG	TTTCCTTCCA	CAGAGTCCGC	450
132	sProProArg	AsnSerAlaM	etHisLeuCy	sPheLeuPro	GlnSerProL	148
					GCTGAAGTTT	
149	euMetPheLy	sAspGlnMet	GlnGlnAspV	alIleMetVa	lLeuLysPhe	165
				00011010E	maaaaaaaaa	EEA
					TGCCCCACTT	
165	ProserAsns	erProvalTn	rhisvalala	ALAASHTHIL	euProHisLe	101
1	03.003.00000	CONCECTO	CA CECA CETE	CAGCCGACTC	TTTGCAGTGA	600
					PheAlaValA	
102	urnitierio	MIGAGIAGII	mrvarime	5502123202		
601	<b>አ</b> ሞአርኔጥርርር እ	CAACACAGTA	GGCCTCAGAG	GAGCTCCAGG	ATACTCCTTG	650
199	snaraTroHi	sAsnThrVal	GlyLeuArqG	lvAlaProGl	yTyrSerLeu	215
	~ z P				_ <b>_</b>	
651	GATCAAGCCC	ACCATCTTCC	CATTGAAATG	GATCCATTAA	TAGCCAATAA	700
					leAlaAsnAs	

# Fig. 1 (sheet 1 of 3)

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701	TTCAGGTGTA	AACAAACGGC	AGATCACAGA	CCTCGTTGAC	CAGAGTATAC	750
232	nSerGlyVal	AsnLysArgG	lnIleThrAs	pLeuValAsp	GlnSerIleG	248
751	AAATCAATGC	ACATTGTTTT	GTGGTAACAG	CAGATAATCG	CTATATTCTT	800
249	lnIleAsnAl	aHisCysPhe	ValValThrA	laAspAsnAr	gTyrIleLeu	265
				COMMAN MA CMA	CACAAACACC	950
801	ATCTGTGGAT	TCTGGGATAA	GAGCTTCAGA	GTTTATACTA ValTyrThrT	hrGluThrGl	281
265	ITeCAseTAb	nerrpasply	sserrheard	AGITATIMET	mr Gruimr Gr	202
251	<b>CA A A TOTCA COT</b>	CAGATTGTAT	TTGGCCATTG	GGATGTGGTC	ACTTGCTTGG	900
282	vLvsLeuThr	GlnIleValP	heGlyHisTr	pAspValVal	ThrCysLeuA	298
901	CCAGGTCCGA	GTCATACATT	GGTGGGGACT	GCTACATCGT	GTCCGGATCT	950
299	laArgSerGl	uSerTyrIle	GlyGlyAspC	ysTyrIleVa	lSerGlySer	315
				> cmacaaaaa	3.003.003.0003.00	1000
951	CGAGATGCCA	CCCTGCTGCT	CTGGTACTGG	AGTGGGCGGC SerGlyArgH	ACCATATCAT	331
315	Argaspalar	urrenrenre	diffiditf	Sergrykrym	13111311011	<b>552</b>
1001	AGGAGACAAC	CCTAACAGCA	GTGACTATCC	GGCACCAAGA	GCCGTCCTCA	1050
332	eGlvAspAsn	ProAsnSerS	erAspTyrPr	oAlaProArg	AlaValLeuT	348
1051	CAGGCCATGA	CCATGAAGTT	GTCTGTGTTT	CTGTCTGTGC	AGAACTTGGG	1100
349	hrGlyHisAs	pHisGluVal	ValCysValS	erValCysAl	aGluLeuGly	365
				maaammamaa	3.03.003.003.00	1150
1101	CTTGTTATCA	GTGGTGCTAA	AGAGGGCCCT	TGCCTTGTCC CysLeuValH	igThrTleTh	381
365	renvarries	ergryarany	SGIGGIALTO	Cybbearain	#D2000	-
1151	TGGAGATTTG	CTGAGAGCCC	TTGAAGGACC	AGAAAACTGC	TTATTCCCAC	1200
382	rGlyAspLeu	LeuArgAlaL	euGluGlyPr	oGluAsnCys	LeuPheProA	398
1201	GCTTGATATC	TGTCTCCAGC	GAAGGCCACT	GTATCATATA	CTATGAACGA	1250
399	rgLeuIleSe	rValSerSer	GluGlyHisC	ysIleIleTy	rTyrGluArg	415
44-4		am > mmma > 0	CAMMA AMCCC	AAACTTTTGG	СФСХХХФССХ	1300
1251	GGGCGATTCA	GTAATTTCAG	rTleAsnGlv	LysLeuLeuA	laGlnMetGl	431
#T2	Gryargenes	erasm nebe				
1301	GATCAATGAT	TCAACACGGG	CCATTCTCCT	GAGCAGTGAC	GGCCAGAACC	1350
432	ulleAsnAsp	SerThrArgA	lalleLeuLe	uSerSerAsp	GlyGlnAsnL	448
1351	TGGTCACCGG	AGGGGACAAT	GGGGTAGTAG	AGGTCTGGCA	GGCCTGTGAC	1400
449	euValThrGl	yGlyAspAsn	GlyValValG	luValTrpGl	natacysasp	405
	mmo. 1	mom3 c3 mmm3	3 <i>0000000</i> 300	TGATGCTGGC		1450
	TTCAAGCAAC PheLysGlnL		ACCUIGNIG	IGNIGCIGGC	MAJONDALLA	
400	- Hanaserun	entlite				

# Fig. 1 (sheet 2 of 3)

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1451 TGGACTTGTC CCATGACCAG AGGACTCTGA TCACTGGCAT GGCTTCTGGT 1500
1501 AGCATTGTAG CTTTTAATAT AGATTTTAAT CGGTGGCATT ATGAGCATCA 1550
1551 GAACAGATAC TGAAGATAAA GGAAGAACCA AAAGCCAAGT TAAAGCTGAG 1600
1601 GGCACAAGTG CTGCATGGAA AGGCAATATC TCTGGTGGAA AAAATTCGTC 1650
1651 TACATCGACC TCCGTTTGTA CATTCCATCA CACCCAGCAA TAGCTGTACA 1700
1701 TTGTAGTCAG CAACCATTTT ACTTTGTGTG TTTTTTCACG ACTGAACACC 1750
1751 AGCTGCTATC AAGCAAGCTT ATATCATGTA AATTATATGA ATTAGGAGAT 1800
1801 GTTTTGGTAA TTATTTCATA TATTGTTGTT TATTGAGAAA AGGTTGTAGG 1850
1851 ATGTGTCACA AGAGACTTTT GACAATTCTG AGGAACCTTG TGTCCAGTTG 1900
1901 TTACAAAGTT TAAGCTTTGA ACCTAACCTG CATCCCATTT CCAGCCTCTT 1950
1951 TTCAAGCTGA GAAAAAAAA AAAAAAAA 1979

Fig. 1 (sheet 3 of 3)

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1	CGAG	GGA	GGA	GGA	AGA	CCA	AGA	CAT	ACA	GGG	AGA	AAT	'CAG	TCA	TC
	R	E	E	E	D	Q	D	I	Q	G	E	I	S	H	P
46	CTGA	TGG	AAA	.GGT	'GGA	AAA	.GGT	TTA	TAA	GAA	TGG	GTG	CCG	TGT	TA
						K									
91	TACT	'GTT	TCC	CAA	TGG	AAC	TCG	AAA	GGA	AGT	GAG	TGC	AGA	TGG	GA
						T									
136	AGAC	CAT	CAC	TGT	CAC	TTT	CTI	TAA	TGG	TGA	CGT	GAA	.GCA	GGT	'CA
	T	I	T	V	T	F	F	N	G	D	v	K	Q	V	M
181	TGCC	AGA	CCA	AAG	AGT	GAT	CTA	CTA	CTA	TGC	AGC	TGC	CCA	GAC	CA
	P	D	Q	R	V	I	Y	Y	Y	A	A	A	Q	T	T
226	CTCA	CAC	GAC	ATA	.ccc	GGA	GAN	CTG	GGA	AGT	CTT	ACA	TTT	CTC	AA
	H	T	T	Y	P	E	X	W	E	V	L	H	F	S	S
271	GTGG	ACA	AAT	AGA	AAA	ACA	TTA	CCC	AGA	TGG	AAG	AAA	AGA	AAT	CA
	G	Q	I	E	K	H	Y	P	D	G	R	K	E	I	T
316	CGTT	TCC	TGA	CCA	GAC	TGT	TAA	AAA	CTT	ATT	TCC	TGA	TGG	ACA	AG
	F	P	D	Q	T	V	K	N	L	F	P	D	G	Q	E
361	AAGA	AAG	CAT	TTT	CCC	AGA	TGG	TAC	AAT	TGT	CAG	AGT	ACA	ACG	TG
	E	S	I	F	P	D	G	T	I	v	R	V	Q	R	D
406	ATGG	CAA	CAA	ACT	CAT	AGA	GTT	TAA	TAA	TGG	CCA	AAG	AGA.	ACT.	AC
	G	N	K	L	I	E	F	N	N	G	Q	R	E	L	H
451	ATAC	TGC	CCA	GTT	CAA	GAG	ACG	GGA	ATA	.ccc	AGA	TGG	CAC'	TGT	TA
	T	A	Q	F	K	R	R	E	Y	P	D	G	T	V	K
496	AAAC	CGT	ATA	TGC.	AAA	CGG	TCA	TCA	AGA	AAC	GAA	GTA	CAG	ATC	CG
	T	V	Y	A	N	G	H	Q	E	T	K	Y	R	S	G
541	GTCG	GAT.	AAG.	AGT'	TAA	GGA	CAA	GGA	GGG	TAA	TGT(	GCT.	AAT(	GGA	CA
	R	I	R	v		D	K	E	G			L	M	D	T
586	CGGA E		GTG.	ACG.	ATC	CTC.	ATG	TGA	TCA	TGA	<b>AGT</b>	AAC.	AGT:	AAC'	TG
531	ACTT	TTT:	ATG'	TTA	AAA	AAT	GTA	CAT	TTA	CTG	TGG	ATT(	CTG!	r <b>t</b> T	AA
576	TTTA	TTG'	rgt:	ATG'	TGT(	GGG	GAA	AAG.	ATT	GGA'	TTC:	raa:	AAT	AAA	AG
721	TTTA	ccc	rg <b>t</b> (	GGC	AAA			_		-					

Fig. 2

### 5/20

1	CT	CCA	AGG	GGC	GGG	CAG	AAG	TTG	GAA	LACA	TGC	:GGC	TGI	'CGG	TCGC
						R									
46	TG	CAG	CAT	CTC	CCA	TGG	CCG	CGI	'ATI	TCG	ĊCG	TAI	'GGG	CCI	CGGT
	С	S	I	S	H	G	R	V	F	R	R.	M	G	L	G
91	CC	CGA	GTC	CCG	CAT	CCA	TCT	GTT	GCG	GAA	CTI	GCT	CAC	AGG	GCTG
٠	P	E	S	R	I	H	L	L	R	N	L	L	T	G	L
136															CGAA
						I									
181	AT														GGGA
	M	R	G	Y	A	E	K	L	I	D .	Y	G	K	L	G
226	GA	CAC	TAA	CGA											CACA
	D	T	N	E	R	A	M	R	M	A	D	F	W	L	T
271	GA														TCGG
	E					P				_					
316															CCCA
	Y			_		G							_		
361															TAAA
	N					R									
406															CCAC
	G					P									
451															CCTC
						Q							_		
496															TCAA
	R	Q	S	Q	E	A	S	N	H	S	S	H	T	A	Q
541	ACZ T		AGG(		TTA.	ACT	GGA'	TCT	GAA	GAG'	TCT	GCA	GCC	CTT.	AATC
	•	•	•	_											
586	AG:	PAC	CCA!	rga:	rca(	CAG	GCC'	TTT(	GGA	GCA	CTT'	TTA	CTC:	rc T	GAGA
631	AG	AC:	rgg/	AGC:	rag:	AGA'	rgt	AAA	ATG	GAC	AGT	CTT	GAT(	GGG	GTTG
676	AG	AAC	CTT(	CTG	GGZ	AGC	CAG	ATG	ACC	CTC'	rct'	rtg(	CAC	AAT	AGAT
721	AAI	\AG	rct?	rta:	rat(	GAA!	PAT	ATA!	PAA	ATT'	rat'	TTA:	rrt'	rtc	CTCC
766	TGT	rgg(	GAT!	rtc:	rggi	AGAI	ATG	AGAI	ATT	ATC	CAA	ATG	CCC	AGT	CTAC
811	CTC	AG	ATAC	TAI	AT:	rca:	rgg	CTT	ATG	CTT(	CTG	GTC	CTT	<b>LA</b>	

Fig. 3

1	AA	TAG	ACC	AGG	CTI	'GGG	CCA	\GA/	\TG#	LGAA	TCI	'GAG	TGC	CAT	TGAG	
	N	R	P	G	L	G	Q	N	E	N	L	S	A	I	E	
46	GG	GAA	AGG	CAA	.GGT	'GGG	GGG	ACI	'GAA	GAC	'ACG	CTG	CTC	TAG	CTGC	
	G	K	G	K	v	G	G	L	K	T	R	C	S	S	C	
91	AA	CGT	ממיי	ርም	בטיויי	ርሞር	ጥርያ	AAC	נטתנ	אכיז	יככי	GAA	CCA	ר איז.	CCAA	
-						S										
136	a C	ሮልጥ	יררז	יררנ	מממני	CCT	CCT	יכרר	י <i>א</i> כי	CAG	מ מיי	CAG	CAC	מיתי	GATG	
130						L										
101	22	3 3 C	~~~	,,,,,	3 CM	3 ma	300		1000				maa	OMO	CCAG	
101						S				-				_		
				_											-	
226						.GAC T									GGTT	
								_					_			
271						GGA D									CATG	
	ц	I	14	£	W	ע	1	Q	٧	л	V	A	7.4	Д	M	
316															GACC	
	I	D	E	G	L	N	H	E	С	K	L	C	S	Q	T	
361															CAGC	
	F	D	S	P	A	K	L	Q	C	H	L	I	E	H	S	
406	TT	CGA	AGG	GAT	GGG	AGG	CAC	CTI	CAA	GTG	TCC	AGT	CTG	CTT	TACA	
	F	E	G	M	G	G	T	F	K	C	P	V	C	F	T	
451	GT:	ATT	TGT	'TCA	AGC	AAA	CAA	GTI	'GCA	GCA	GCA	TAT	ттт	CTC	TGCC	
	V	F	V	Q	A	N	K	L	Q	Q	H	I	F	S	A	
496	CA	TGG	ACA	AGA	AGA	CAA	GAT	ста	TGA	CTG	TAC	ACA	ATG	ጥርር	ACAG	
						K										
541	22	שועוב	th thirt	الماليات	CCA	አአሮ	n C'n	CCT	CCA	CAA	መረ እ	ma c	አአጥ	C 3 C	CCAA	
J=1						T										
E0 <i>6</i>	<b>C</b> 34	a. a	~~	mm >	0m0	<b>~~</b>	~m»	010	man	ama		~~		<b></b> -	mmm	
266	H	CAG S	CAG S	TTA	GTG	CAA	GTA	CAG	TCT	CTC	AAG	GAG.	AAT	TGA	TTTT	
631	GT(	GGC.	ACA	AAA	AGG	GAA(	CAT	GTT	'TTA	.CTC	TTT	GCA	CGA	AAC	TTTC	
676	AT!	rgt	TAA	TGT	ATA	TTA:	TTC	AGA	AAC	ATT	GTA	TTG'	TAC	CAT.	AAAA	
771	c.mi	nam	2 MM	3 mc		amai	7M/		c mm	/ 3 M	CMC	mmm	~~~	~~~	TTGC	
121	CT.	rgr.	ATT	ATC	AAA	CTG.	rrG	GAT	GTT.	CAT	GTG	1.1.1.	GAA	CTT	TTGC	
766	GC	ACC	GGA	TAG	ACC	CCT'	rgt	ATA	TAA	AGT	GTT	GCA	CAT	GTA	TTAT	
811	GT	CT	ርፓር	АТА	СТА	AAA'	rgg	ጥርጥ	TAT	AAA	GAC	AAC	TGC:	ACT'	TGGG	Fig. 4
																/ t t d
856	CCC	C'TA'	TTC	AGG	CAA	GAT'	raa	AAA	AAA	AAA	AAA	GAC'	TAT	GAC	CAAA	(sheet 1 o
						CI	тос		reti	CHI	TT	<b>PI</b> II	IF 2	6)		

901	ATGGCTTAAGATAAAGTATTTTTAAGGAAGAAAGA	TTAAAA	ACA	A
946	CTGTTATACATGAGACTATGGTTGGACTTCCTTTT	CTTTAC	ACT	T
991	AAGCCTAAAATTTCTCCTTTAGGTATATCAGCGCT	TAAATC	CAA	G
1036	ACTATTTTTATTGCTGAAGATTCTTGCAAACCAT	GAAGAG	ATG	T
1081	TCTCACAGAACAGAACCCCCACAGCTGGNATAAGG	CCCGGT	ATA	T
1126	ATATATTTGGTAAAGCCCTTGCAATGTGACAGGGT	AGCATC	ACT.	A
1171	ATATATGCAATAGGTTGTTATGGAGACTGTCAAAG	LAATTTT	TTT	T
1216	TCCCTGGATACATTTGAAGCTTTGAGTGTTCAAGG	TTTTCC	TTA	A
1261	TGATTTCACGCAGCCAAATTCTTGAATCAGTTGAA	CTAACC	TGT.	A
1306	TGTTACTGTTATTAATGTTTACTCTGCAGTCTGAA	.CCTGGA	GAT	T ·
1351	ACTGGAATTGTTTTCCAAGAGGAAATAAATTCAGT	TTACCA	TTA	G
1396	GAAAAAAAAAAA	Fig.	4	(sheet 2 of 2)

1	CGG	CAC	:GA(	<b>3</b> GG(	CAG	GGG	ACC	TGC	TCC	CCZ	ACT	rcci	ACAG	AGC	CGAG			
46	CTG	TTA	CGC	TA?	CTC	GGG	TGC	CTC										
									С	H	H	P	V	С	A			
91	GCC	'AGA	TCI	rgco	CTO	TTA	TCC	TGG	AG"	rcco	CAC	CCZ	AAA	GAG	CCA			
	P	D	L	R	C	Y	P	G	V	P	T	Q	R	S	H			
126	CTA	mee		D 3 C C	א מוויר	יייי	ccc	man	nc-c	א מחר	יחחי	CITIC C	מתחי	·mm/	am Can			
130	-				S					-								
				_	-	_	_	_			_	_	_		_			
181	CTG										-							
	C	Q	P	G	L	ъ	C	P	G	S	V	W	G	C	ע			
226	CCC	'AGC	TGI	\GG7	CAC	:CAA	.GCT	CCA	\GC2	AGC 1	CTC	'AAC	CCA	TGC	GGT			•
	P	A	E	V	T	K	L	Q	Q	L	S	S	H	A	v			
271	CCC	יריתים	ייזיכר	יראר	יארר	יכאכ	CCT	יכפיז	ecc	73 CC	מיי) מינ	rcc i	יייירי	cee	ייארי			
4; I					P													
316	ACA																	
	Q	T	5	5	Q	Ŀ	F	П	V	P	M	ע	L		G			
361	CTG																	
	C	V	I	G	R	Q	G	S	K	I	S	E	I	R	Q			
406	GAT	ነርጥር	יאמני	יממנ	מיאני	ידעידי	ממי	CAT	ירכנ	ica z	יכרז	AGC	ים כם	CCC	ירפר			
200					H													
451	TGG				LTGI V									-				
	G		K	11	٧	•	_		G	5	F	٧	3	_	A			
496	CCT																	
	L	A	Q	Y	L	I	T	A	С	L	E	T	A	K	S			
541	TAC	CTC	TGG	GGG	GAC	GCC	CAG	СТС	:GGC	CCC	:CGC	AGA	CCT	GCC	TGC			
					T													
E0 <i>E</i>	000	OMM.	com c			-	<b>~</b> ~ ~						maa	000	maa			
360	CCC				P										G			
															•			
631	CCT																	
	ъ	1.	G	т	P	Y	A	Ţ	S	Ţ	S	N	F.	I	G			
676	CCT	CAA	GCC	CAT	'GCC	CTT	CTT	GGC	TTT	'ACC	ACC	TGC	TTC	CCC	AGG			
	L	K	P	M	P	F	L	A	L	P	P	A	S	P	G			
721	GCC	ממם	<u>c</u> cc	rann Tann	ייוואיי	CCC.	aca	∕-m->	(T) (T)	יחירים	(C) N P	C a m	CCC	3 <i>0</i> 0	200			
/21					L									AGC A				
766	TAA															⊏:~	E	
					K											Fig.		
811	GCC.	AGG	TAG	CTG	GGG	GAG'	TGG	CAT	GGG	GCA	GGG	GCC	CAG	TTC	TCA	(shee	et 1	of 2
									٠.	eur <sup>®</sup>						,		

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P G S W G S G M G Q G P S S Q

856 GCAGCAGACACTCTGTACAGTTTTTTCAATCCCTGTTTTTGAATA
Q Q T L C T V F S I P V F E

901 AATATTCTCAGCGACCAAAAA

Fig. 5 (sheet 2 of 2)

1	GAGTAGGAAGTGGTGAGTTCGGAGTAGCAGA:	rgg	CCGC	GCT	TGCA
	M	A	A	T.	A

- 46 CCGCTGCCCCGCTCCCCGCACAGTTCAAGAGCATACAGCATCAT
  P L P P L P A Q F K S I Q H H
- 91 CTGAGGACGCTCAGGAGCATGACAAGCGAGACCCTGTGGTGGCT L R T A Q E H D K R D P V V A
- 136 TATTACTGTCGTTTATACGCAATGCAGACTGGAATGAAGATCGAT
  Y Y C R L Y A M Q T G M K I D
- 181 AGTAAAACTCCTGAATGTCGCAAATTTTTATCAAAGTTAATGGAT S K T P E C R K F L S K L M D
- 226 CAGTTAGAAGCTCTAAAGAAGCAGTTGGGTGATAATGAAGCTATT Q L E A L K K Q L G D N E A I
- 271 ACTCAAGAAATAGTGGGCTGTGCATNTTTGGAGAATTATGCTTTG T Q E I V G C A X L E N Y A L
- 316 AAAATGTTTTTGTATGCAGACAATGAAGATCGTGCTGGACGATTT
  K M F L Y A D N E D R A G R F
- 361 CACAAAACATGATCAAGTCCTTCTATACTGCAAGTCTTTTGATA H K N M I K S F Y T A S L L I
- 406 GATGTCATAACAGTATTTGGAGAACTCACTGATGAAAATGTGAAA D V I T V F G E L T D E N V K
- 451 CACAGGAAGTATGCCAGATGGAAGGCAACATACATCCATAATTGT H R K Y A R W K A T Y I H N C
- 496 TTAAAGGAATGGGGAGACTCCTCAAGCAGGCCCTGTTGGGAATTG L K E W G D S S S R P C W E L
- 541 AAGAAGATAATGATATTGAAGAAATGAAGATGCTGGAGCAGCCT K K I M I L K K M K M L E Q P
- 586 CTCTGCCCACTCAGCCAACTCAGCCATCATCTTCAACTTATG L C P L S Q L S H H H L Q L M
- 631 ACCCAGCAACATGCCATCAGGCAACTATACTGGAATACAGATTCC T Q Q H A I R Q L Y W N T D S
- 676 TCCGGGTGCACACGTTCCAGCTAATACACCAGCAGAAGTGCCTCA S G C T R S S
- 721 CAGCACAGGTGTAGCAAGTAATACTATCCAACCTACTCCACAGAC
- 766 TATACCTGCCATTGATCCCGCACTTTTCAATACAATTTCCCAGGG
- 811 GGATGTTCGTCTAACCCAGAAGGCTTT

1	GGA	GTA	TGA	GAT	GAA	ACG	AAT	GGC	AGA	GAA	TGA	GCT	GAG	CCG	GTC
				M			M								S
46.	AGT	AAA	TGA	GTT	TCT	GTC	CAA	GCT	GCA	AGA	TGA	CCI	CAA	GGA	GGC
													K		
91	AAT	GAA	TAC												GAG
	M	N	T	M	M	C	S	R	С	Q	G	K	H	R	R
136	GTT	TGA	AAT	GGA	CCG	GGA	ACC	TAA	GAG	TGC	CAG	ATA	.CTG	TGC	TGA
													C		
181	GTG	TAA	TAG	GCT	GCA	TCC	TGC	TGA	GGA	AGG	AGA	CTT	TTG	GGC	AGA
	C	N	R	L	H	P	A	E	E	G	D	F	·W	A	E.
226	GTC	AAG	CAT	GTT	GGG	CCT	CAA	GAT	CAC	СТА	CTT	TGC	ACT	GAT	GGA
		S		L	G	L	K	I	T	Y	F	A	L	M	D
271	TGG	222	ርርጥ	CT A	TCA	CAT	CAC	ACA	GTG	ccc	ጥርር	ATG	CCA	GCG	ጥርጥ
47.1	G												Q		
316	AGG	ጥልጥ	СТС	ררר	a Ca	ጥልሮ	CCA	CAG	እ <i>ር</i> ጥ	ccc	ርጥ አ	ጥርት አ	СЪТ	כיתיכ	Δጥጥ
310	G	I	s	P	D	T	Н	R	·V	P	Y	H	I	S	F
361	TGG	יייינייוי	ጥርረ	ייעבי	ጥሮሮ	a cc	<sub>ር</sub> ልሮ	CAG	) CC	ccc	CCA	GAG	AGC	CAC	CCC
301													A		
406	AGA	mcc.	CCC	mcc	መረረ	<b>ም</b> ር አ	ጥርጥ	ጥሮል	CCA	ւերեր	արդ Մարդ	GAG	ጥሮር	ሮልጥ	Cuturp 1
400													R		
451	TCA	n cm	200	~~~	300	CCN	C N III	ccc	מו א	ccc	אאר	thirth:	ananan.	CCA	CCT
43I	Q	V	P	P	G G	Q	M	P	M	G	T	S	L	Q	L
496	CCT	CAG	CCT	GCC	CCT	GGA	GCC	GCT	GCA	GCC	TCT	AAG	CCC.	AAC.	AGC
													P		
541	ACA	CTA	CCC	AAG	GGA	GAA	GCC	AAA	ССТ	AAG	CGG	CGG	AAG	AAA	GTG
	Q	Y	P	R	E	K	P	N	L	S	G	G	R	ĸ	
586	AGG.	AGG	CCC'	TTC	CAA	CGT	TGA	TGC	CCC	TTC'	TCT	TTC	CTC.	AAA	TCA
631	ATG	TCA	GGG	AGT	CAA	AAG	GGC	TGT.	AGC	ACA(	GGA	TGG	AGT	TTG	ATT
676	TAT	CCC'	TCC'	rcc	CCC	AAC.	ACC	TAG	GAA	CTG	AAT	CTT	TTT	CTT	TTT
721	ATT	TTT'	TGA	GAT	GGA	GTC	TTG	CTC	TGT	TGC	CCA	G			

Fig. 7

1	GTT	TAA	AGC	TGA	GAA	CGC	ATC	TTT	AGC	TAA	ACI	TCG	CAT	TGA	ACG
	F	K	A	E	N	A	S	L	A	K	L	R	I	E	R
				~											
46	AGA														CGA
	E	S	A	L	E	K	L	R	K	E	I	A	D	F	E
91	ACA	ACA	GAA	AGC	AAA	AGA	ATT	AGC	TCG	AAT	AGA	AGA	GTT	TAA	AAA
										I			F		K
	Q	Q	V	A	V	£	u	A	K	_	Ŀ	E	F	K	A
136	GGA	GGA	GAT	GAG	GAA	GCT	'ACA	AAA	GGA	ACG	TAA	AGT	TTT	<b>TGA</b>	AAA
	E	F.	M	R	ĸ	T.	0	ĸ	E	R	ĸ	v	न	E	K
							~								
181	GTA														
	Y	T	T	A	A	R	T	F	P	D	K	K	E	R	E
226	AGA	AAT	ACA	GAC	TTT	AAA	ACA	.GCA	AAT	'AGC	AGA	TTT	ACG	GGA	AGA
	E	Т	0	T	T.	K	0	0	T	A	n	T.	R	E.	D
		_	×	•	_	••	×	×	_	••		-		_	
271	TTT	GAA	AAG	AAA	GGA	GAC	CAA	ATG	GTC	AAG	TAC	ACA	CAG	CCG	TCT
										S					L
		11	11	11	13	*		**		5	•	**			
316	CAG	AAG	CCA	GAT	ACA	AAT	GTT	AGT	CAG	AGA	GAA	CAC	AGA	CCT	CCG
	R	S	0	I	0	M	L	v	R	E	N	T	D	L	R
		_	π.		*		_			_				_	
361	GGA	מממ	מע ג	222	аст	CAT	CCA	AAG	ידיים ב	ירכפ	аст	CCA	ጥርጥ	CTC	CAA
<b>301</b>															
	E	E	Τ.	K	٧	М	E	K	F	R	ш	ע	V	W	K
406	GAG	AGC	AGA	AGC	CAT	AGA	GAG	CAG	CCT	CGA	GGT	GGA	GGA	GGA	GGG
	R	A	E	A	I	E	S	S	L	E	V	E	E	E	G
			_		_		_	_	_	_	_		_	_	
451	CAA	GCT	TGC	GAA	CAC	ATC	TGT	TCG	ATT	TCA	AAA	CAG	TCA	GAT	TTC
	77	T.	7	NT.	TP.		37	- D	T	Q	N	ď		T	-
		ш	A	TA	_	3	•		T.	×	74	5	¥	-	3
496	TTC	AGG	AAC	CCA	GGT	AGA	AAA	ATA	CAA	GAA	AAA	TTA	TCT	TCC	AAT
										K					
	.5	G	-	×	٧	E		•	17	11	7.4	_		_	1.7
541	GCA	AGG	TAA	GAG	GCT	GCA	TGA	тст	ттт	TAT	AAA	ACA	TTT	CAG	AAT
										I					М
	V	G	v	K	Ţ	п	ע	ш	Ľ	T	77	п	Ľ	R	11
E 0 6	GTA	* ~~	3 3 M		<b>~~</b> -	mmm	2002		220	mm =	3 M 3		(1 ) M	mmæ	mm »
200	GTA	טטמ	VVI.	MAA	CAA	T.T.T.	MIA		MAC	TTA	WIA	WW	CMT	TTC	TTA
631	አመአ	3 3 M	CTT	Mmm	<b>~22</b>	C R M	mm~	2.2							

Fig. 8

1	CTTC	TTC	TCT	CTT	TGC	TTC	AGA	CTG	GCC	CGG	GAG	CAA	GCG	CGA	GT
_	S	S	L	F	A	S	Ď	W	P	G	S	K	R	E.	C
46	GTGC	GAA	CTG	CAG	AGT	GGG	AAC	CAG	CAG	CTG	GAG	GAG	CAG	CGG	GT
	A	N	C	R	V	G	T	S	S	M.	R	S	S	G	W
91	GGAG	CTG	GTG	GAA	AGA	CTG	CAG	GCC	ATG	СТG	CAG	GCC	CAC	TGG	GA
	S	W	W	K	D	C	R	P	C.	С	R	P	T	G	M
136	TGAG	GCC	AAC	CAG	CTG	CTC	AGC	ACC	ACT	CTC	CCG	CÇG	ccc	AAC	CC
	R	P	T	S	C	S	A	P	L	S	R	R	P	T	L
81	TCAA	GCT	CCI	CCI	GAT	GGA	CCC	TCC	AGC	CCC	GGG	CCT	CAG	GAG	CC
	K	L	L	L	M	D	P	P	A	P	G	L	R	S	P
226	CGAG	AAG	GAG	GAG	AGG	AGG	GTN	TGG	ACT	ATG	CCT	CCC	ATG	GCC	GT
	R	R	R	R	G	G	X	G	L	C	L	P	W	P	M
271	GGCC	'TGA	AGC	CTG	TAT	TGC	:AGC	AGA	GCC	GGG	AAG	CAA	GGG	ACG	AG
	P	E	A	C	I	A	A	E	P	G	S	K	G	R	A
316	CTAC	:CTG	GAC	CGC	CTC	CTG	TTC	TTI	'GCA	GTI	'CCT	CCT	CAG	ATC	TT
	T	W	S	A	S	C	S	L	Q	F	L	L	R	S	

361 AGCCTCCTGTTGGGCCCCTCTTTTCA

Fig. 9

1	GCT	CTA	CCG	GCC	'AG!	AAA(	GAC	AGC	CAI	'GG'	[CA]	AGTO	<b>F</b> TGC	CT	\GA	•			
46	GGC'	rgce	CTI	ניסטי	CAC	CT	ACTO	3GC(	BAGO	GT(									
0.1	688												A	_	_				
31	GCC(	E E																	
136	CGG	CGA	TCC	AGG	TG	LAG(	CTC	3CGG	ACA	GCG	AGA	MCG	AGG	AGA	ΥA				
	A	D	P	G	E	A	C	G	Q	R	X	R	G	D	R				
181	GAA	laci	CTI	CGI	'GGG	CA	rgç1	CAA	CAA	GCI	ACA	GTC	CGA	GGA	CG				
		L																	
226	ACG1	IGCG R																	
271	CCAT													_					
4,1	I	L	R	G	P	D.	G	N	S	K	G	C	A	F	V				
316	TGA	GTA	CTC	CTC	CCA	CGC	CGA	\GGC	:CAG	GCC	GCC	ATT	CAA	.CGC	GC				
	K	Y	S	S	H	A	E	A	R	P	P	F	N	A	L				
361	TACA																		
		G																	
405	TCAA K	GTT. F	CGC A	CGA D	CAC T	CGA D	CAA K	LGGA E	.GCG R	CAC T	GAT M	GCG R	GCG R	AAT M	GC				
451	AGCA														_				
		M																	
496	CTTT																		
	F	G	A	Y	G	A	Y	A	Q	A	L	M	Q	Q	Q				
541	AAGC	GGC A	CCT	GAT	GGC	ATC	GGT	CGC	GCA	GGG	CGG	CTA	CCT	GAA	TC				
50 <i>C</i>																			
200	CCAT M	A	A A	F	CGC A	TGC A	CGC A	CCA Q	GAT M	gca Q	GCA Q	GAT M	GGC A	GGC( A	CC				
631	TCAA	CAT	GAT	ATG	GGC	TGG	CAG	CCG	CAC	CTA'	TGA	CCC	CAA	CCT	CA				
		M	I	W	A	G	S		T	Y	D	P	N	L	R				
676	GGTG												TGC	CTA	GC.				
		Q									_	_	A						
721	ATCC	CAT	CCC	CA.	rg T	GGG	TGA	ACG	GCT'	rca(	CGG	GCC'	rcc	CCC:	rc				
766	AGGC	CAA!	rgg(	3CA(	GCG'	rgc'	TGC	GGA	ACG?	rgt	GTN:	rgc	CAA!	rgn(	3C				
811	ATTC.	ACC	GTA(	CGCZ	AGC	ACA	GAG	ccc	CAC	3GC2	AGC	CGA	ccc	CGT	3C	Fig.	10	)	
856	AGCA	GGC	CTAC	GC	rggi	AGT'	TGC	AGA	GTAT	rcg2	AGG	ACG	rgco	CTAC		(shee			2)
																			,

901	CTGCTGCCTATGGTCAGATTAGCCAGGCCTTTCNTCAGCGACGGC	
946	GAATGATTCCCAGCANAGAGAGAGGGCCGTAGGTGTTATTTTTC	3
991	CGGCGCGTCGCGGAGTTGTGGACGACGTGGAAGAGCTGCCTGTC	3
1036	GTTGGGGTTGAAGATGAGACGAAACGATCTCGATAGGGTTACAT	ŗ
1081	CTGAGGCGAGGGCGGTTGGGTAAGAGTTCCGAATTTGGTGTGCTC	
1126	GGCACGATGGAGACAGGCGAGAATAACATTCTATATGGAGGGGT	r
1171	AACAAGAGAAAGGTTGTGTTATAGAGGGGGAGTAAATGAAAAACA	<b>V</b>
1216	TATGGGGAGCCTCTTCTTCCTC	Fig. 10
		(sheet 2 of 2)

- 1 CCGGAACGGCTGAGGAAGGGCCCGTCCCGCCCTTCCCCGGCGCGCCC PERLRKGPSRLPRRA
- 46 ATGGAGCCCCGGGCGGTTGCAGAAGCCGTGGAGACGGGTGAGGAG M E P R A V A E A V E T G E E
- 91 GATGTGATTATGGAAGCTCTGCGGTCATACAACCAGGAGCACTCC D V I M E A L R S Y N Q E H S
- 136 CAGAGCTTCACGTTTGATGATGCCCAACAGGAGGACCGGAAGAAA Q S F T F D D A Q Q E D R K K
- 181 CTGGCGGAGCTGCTGGTCTCCGTCCTGGAACAGGGCTTGCCACCC L A E L L V S V L E Q G L P P
- 226 TCCCACCGTGTCATCTGGCTGCAGAGTGTCCGAATCCTGTCCCGG S H R V I W L Q S V R I L S R
- 271 GACCGCAACTGCCTGGACCCGTTCACCACCGCCAAAACCTGCAGC D R N C L D P F T T A K T C S
- 316 CTAGCCTGCTTTGCTGACATCTTTGTCTTTGAGGGTCCNTCCCAA L A C F A D I F V F E G P S Q

Fig. 11

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## DEMONSTRATION OF THE LYST:IP INTERACTION

	LYST FORW	LYST REV	LYST-2
14-3-3 protein	+	+	+
Casein kinase II beta SU	+	+	-
B 14-3-3 (HS1)	•	+	-
Calmodulin	•	+	•
Efs	•	+	•
Importin beta subunit	•	+	-
Estrogen receptor related protein	-	+	•
Imogen 38	-	+	•
Atrophin-l	•	+	•
DGS-I	-	+	-
KIAA0607 gene (Norbin)	-	+	-
BMK1 alpha kinase	-	+	
OPA-containing protein	-	+	-
M4 protein	•	+ -	•
Hrs	+	-	-
OS-9 Precursor	+	-	•
Troponin I	+	-	-
GBDR-1	+		<u> </u>
KBO7	+	-	-
Fte-1	+		-
HBF-G2	-	<u> </u>	+
XAP-4	-	-	+
LIP1 (homologies to Tcp 10A)	-	+	<u> </u>
LIP2 (homologies to ribosomal protein L17)	-	+	•
LIP3 (homologies to Roaz protein)	-	+	-
LIP4 (homologies to hnRNP-E2)	-	+	•
LIP5	-	+	-
LIP6 (homologies to viral Ns2-3 protein)	-	+	-
LIP7 (homologies to Tcp 10A)	-	+	-
LIP8 ((homologies to KAP4L protein))	-	+	-
LIP9 (homologies to Xenopus etr-1)		+	-
LIP-10	+	-	-
CDK2		1 -	N.D.
Retinoblastoma	-	N.D.	N.D.
p27(Kip1)	N.D.	<del>                                     </del>	N.D.
RGL-2	N.D.	-	N.D.
Vector control		<del>                                     </del>	<del>                                     </del>
7 55501 60111.01	<del></del>	٠	<del></del>

Fig. 12

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1 TTCCCGGGTCGACCCACGCGTCCGGGTGGCCAAGGGGACTCTCTCCATCACCACGACAGA SerArgValAspProArgValArgValAlaLysGlyThrLeuSerIleThrThrGl

- 61 AATCTACTTCGAGGTAGATGAGGATGATTCTGCCTTCAAGAAGATCGACACGAAAGTTCT ulleTyrPheGluValAspGluAspAspSerAlaPheLysLyslleAspThrLysValLe
- 121 TGCATACACTGAGGGACTTCACGGAAAATGGATGTTCAGCGAGATACGAGCTGTATTTTC uAlaTyrThrGluGlyLeuHisGlyLysTrpMetPheSerGluIleArgAlaValPheSe
- 181 AAGACGTTACCTTCTACAAAACACTGCTTTGGAAGTATTTATGGCAAACCGAACCTCAGT rArgArgTyrLeuLeuGlnAsnThrAlaLeuGluValPheMetAlaAsnArgThrSerVa
- 241 TATGTTTAATTTCCCTGATCAAGCAACAGTAAAAAAGTTGTCTATAGCTTGCCTCGGGT lMetPheAsnPheProAspGlnAlaThrValLysLysValValTyrSerLeuProArgVa
- 301 TGGAGTAGGGACCAGCTATGGTCTGCCACAAGCCAGGAGGATATCATTGGCCACTCCTCG lGlyValGlyThrSerTyrGlyLeuProGlnAlaArgArgIleSerLeuAlaThrProAr
- 361 ACAGCTTTATAAATCTTCCAATATGACTCAGCGCTGGCAAAGAAGGGGAAATTTCAAACTT gGlnLeuTyrLysSerSerAsnMetThrGlnArgTrpGlnArgArgGluIleSerAsnPh
- 421 CGAATATTTGATGTTCCTTAATACTATTGCAGGACGGACATATAATGATCTGAACCAATA eGluTyrLeuMetPheLeuAsnThrIleAlaGlyArgThrTyrAsnAspLeuAsnGlnTy
- 481 TCCAGTGTTTCCGTGGGTGTTAACCAACTATGAATCAGAAGAGTTGGACCTGACTCTTCC rProValPheProTrpValLeuThrAsnTyrGluSerGluGluLeuAspLeuThrLeuPr
- 541 AGGAAACTTCAGGGATCTATCAAAGCCAATTGGTGCTTTGAACCCCAAGAGAGCTGTGTT oGlyAsnPheArgAspLeuSerLysProlleGlyAlaLeuAsnProLysArgAlaValPh
- 601 TTATGCAGAGCGTTATGAGACATGGGAAGATGATCAAAGCCCCACCCTACCATTATAATAC eTyrAlaGluArgTyrGluThrTrpGluAspAspGlnSerProProTyrHisTyrAsnTh
- 661 CCATTATTCAACAGCAACATCTACTTTATCCTGGCTTGTTCGAATTGAACCTTTCACAAC rHistyrSerThrAlaThrSerThrLeuSerTrpLeuValArgIleGluProPheThrTh
- 721 CTTCTTCCTCAATGCAAATGATGGAAAATTTGATCATCCAGATCGAACCTTCTCATCCGT rPhePheLeuAsnAlaAsnAspGlyLysPheAspHisProAspArgThrPheSerSerVa
- 781 TGCAAGGTCTTGGAGAACTAGTCAGAGAGATACTTCTGATGTAAAGGAACTAATTCCAGA lAlaArgSerTrpArgThrSerGlnArgAspThrSerAspValLysGluLeuIleProGl
- 841 GTTCTACTACCAGAGATGTTTGTCAACAGTAATGGATATAATCTTGGAGTCAGAGA uPheTyrTyrLeuProGluMetPheValAsnSerAsnGlyTyrAsnLeuGlyValArgGl
- 901 AGATGAAGTAGTGGTAAATGATGTTGATCTTCCCCCTTGGGCAAAAAAACCTGAAGACTT uAspGluValValValAsnAspValAspLeuProProTrpAlaLysLysProGluAspPh
- 961 TGTGCGGATCAACAGGATGGCCCTAGAAAGTGAATTTGTTTCTTGCCAACTTCATCAGTG eValArgIleAsnArgMetAlaLeuGluSerGluPheValSerCysGlnLeuHisGlnTr

Fig. 13 (sheet 1 of 3)

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	19720
1021	GATCGACCTTATATTTGGCTATAAGCAGCGAGGACCAGAAGCAGTTCGTGCTCTGAATGT plleAspLeullePheGlyTyrLysGlnArgGlyProGluAlaValArgAlaLeuAsnVa
1081	TTTTCACTACTTGACTTATGAAGGCTCTGTGAACCTGGATAGTATCACTGATCCTGTGCT lPheHisTyrLeuThrTyrGluGlySerValAsnLeuAspSerIleThrAspProValLe
1141	CAGGGAGGCCATGGAGGCACAGATACAGAACTTTGGACAGACGCCATCTCAGTTGCTTATuArgGluAlaMetGluAlaGlnIleGlnAsnPheGlyGlnThrProSerGlnLeuLeuIl
1201	TGAGCCACATCCGCCTCGGAACTCTGCCATGCACCTGTGTTTCCTTCC
1261	CATGTTTAAAGATCAGATGCAACAGGATGTGATAATGGTGCTGAAGTTTCCTTCAAATTC uMetPheLysAspGlnMetGlnGlnAspVallleMetValLeuLysPheProSerAsnSe
1321	TCCAGTAACCCATGTGGCAGCCAACACTCTGCCCCACTTGACCATCCCCGCAGTGGTGAC rProValThrHisValAlaAlaAsnThrLeuProHisLeuThrIleProAlaValValTh
1381	AGTGACTTGCAGCCGACTCTTTGCAGTGAATAGATGGCACAACACAGTAGGCCTCAGAGGrValThrCysSerArgLeuPheAlaValAsnArgTrpHisAsnThrValGlyLeuArgGl
1441	AGCTCCAGGATACTCCTTGGATCAAGCCCACCATCTTCCCATTGAAATGGATCCATTAAT yAlaProGlyTyrSerLeuAspGlnAlaHisHisLeuProIleGluMetAspProLeuIl
1501	AGCCAATAATTCAGGTGTAAACAAACGGCAGATCACAGACCTCGTTGACCAGAGTATACA eAlaAsnAsnSerGlyValAsnLysArgGlnIleThrAspLeuValAspGlnSerIleGl
1561	AATCAATGCACATTGTTTTTGTGGTAACAGCAGATAATCGCTATATTCTTATCTGTGGATT nIleAsnAlaHisCysPheValValThrAlaAspAsnArgTyrIleLeuIleCysGlyPh
1621	CTGGGATAAGAGCTTCAGAGTTTATACTACAGAAACAGGGAAATTGACTCAGATTGTATT eTrpAspLysSerPheArgValTyrThrThrGluThrGlyLysLeuThrGlnIleValPh
	TGGCCATTGGGATGTGGTCACTTGCTTGGCCAGGTCCGAGTCATACATTGGTGGGGACTG eGlyHisTrpAspValValThrCysLeuAlaArgSerGluSerTyrIleGlyGlyAspCy
1741	CTACATCGTGTCCGGATCTCGAGATGCCACCCTGCTGCTCTGGTACTGGAGTGGGCGGCA styrllevalserGlySerArgAspAlaThrLeuLeuLeuTrpTyrTrpSerGlyArgHi
1801	CCATATCATAGGAGACAACCCTAACAGCAGTGACTATCCGGCACCAAGAGCCGTCCTCAC sHisIleIleGlyAspAsnProAsnSerSerAspTyrProAlaProArgAlaValLeuTh
	AGGCCATGACCATGAAGTTGTCTGTGTTTTCTGTCTGTGCAGAACTTGGGCTTGTTATCAG rGlyHisAspHisGluValValCysValSerValCysAlaGluLeuGlyLeuValIleSe
1921	TGGTGCTAAAGAGGGCCCTTGCCTTGTCCACACCATCACTGGAGATTTGCTGAGAGCCCT

Fig. 13 (sheet 2 of 3)

SUBSTITUTE SHEET (RULE 26)

rGlyAlaLysGluGlyProCysLeuValHisThrIleThrGlyAspLeuLeuArgAlaLe

1981 TGAAGGACCAGAAAACTGCTTATTCCCACGCTTGATATCTGTCTCCAGCGAAGGCCACTG uGluGlyProGluAsnCysLeuPheProArgLeuIleSerValSerSerGluGlyHisCy

#### 20/20

2041	TATCATATACTATGAACGAGGGCGATTCAGTAATTTCAGCATTAATGGGAAACTTTTGGCsIleIleTyrTyrGluArgGlyArgPheSerAsnPheSerIleAsnGlyLysLeuLeuAl
2101	TCAAATGGAGATCAATGATTCAACACGGGCCATTCTCCTGAGCAGTGACGGCCAGAACCT aGlnMetGluIleAsnAspSerThrArgAlaIleLeuLeuSerSerAspGlyGlnAsnLe
2161	GGTCACCGGAGGGGACAATGGGGTAGTAGAGGTCTGGCAGGCCTGTGACTTCAAGCAACTuValThrGlyGlyAspAsnGlyValValGluValTrpGlnAlaCysAspPheLysGlnLe
2221	GTACATTTACCCTGGATGTGATGCTGGCATTAGAGCAATGGACTTGTCCCATGACCAGAGUTyrlleTyrProGlyCysAspAlaGlylleArgAlaMetAspLeuSerHisAspGlnAr
2281	GACTCTGATCACTGGCATGGCTTCTGGTAGCATTGTAGCTTTTAATATAGATTTTAATCGGTAFLeulleThrGlyMetAlaSerGlySerIleValAlaPheAsnIleAspPheAsnAr
2341	GTGGCATTATGAGCATCAGAACAGATACTGAAGATAAAGGAAGAACCAAAAGCCAAGTTAGTT
2401	AAGCTGAGAGCACAAGTGCTGCATGGAAAGGCAATATCTCTGGTGGAAAAAACTCGTCTA
2461	CATCGACCTCCGTTTGTACATTCCATCACCCCAGCAATAGCTGTACATTGTAGTCAGCA
2521	ACCATTTACTTTGTGTGTTTTTTCACGACTGAACACCAGCTGCTATCAAGCAAG
2581	ATCATGTAAATTATATGAATTAGGAGATGTTTTGGTAATTATT
2641	TTGAGAAAAGGTTGTAGGATGTGTCACAAGAGACTTTTGACAATTCTGAGGAACCTTGTG
2701	TCCAGTTGTTACAAAGTTTAAGCTTTGAACCTAACCTGCATCCCATTTCCAGCCTCTTTT

2761 CAAGCTGAGAAAAAAAAAAAAAAA

Fig. 13 (sheet 3 of 3)

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